

Europe in trouble over aviation

Europe's failure to make a common market of itself is typified by the continuing muddle over civil air transport policy. The time has come to change that.

THE European Community is miscalled the "common market". So much is clear from the evidence that repeatedly comes to light of how its tolerance of restraints on free trade are crippling for the economy of Europe (see, for example, *Nature* 322, 2; 1986). But most of the time, the scale of this self-inflicted damage is hard to assess. Even the brashest of economists would be at a loss to tell what price Europe pays for its decision to forgo the benefits of a sensible division of labour among competing companies in fields such as electronics or general manufacturing. But in air transport the disbenefits are conspicuous.

Most European governments maintain civil airlines ostensibly as a means of enabling private persons to move from one place to another, but then come to regard them as extensions of the national persona, along with their national anthems and national flags. Europeans pay for these conceits in two ways: by extra taxes which allow governments to subsidize national airlines, and then by the higher fares which also partly compensate for inefficiency. It is especially scandalous that the European Commission should for so long have tolerated a state of affairs that cramps what should be one of the essential elements of the Europe it seeks, people's freedom to move about. It is similarly outrageous that the benefits of a now-traditional technology should be so widely withheld from 300 million people.

Now, luckily, some governments are becoming restive, with the Commission showing signs of waking up to its responsibilities. Motives are, inevitably mixed. The British government, which has been pressing for reform for the best part of three years, is eager that its own nationalized airline, which has been forced by the deprivations of recent years to become efficient and which is about to be sold as a going concern, should be able to compete more freely for the untapped market in European air travel. It would also be a legislative convenience if the government, when "privatizing" British Airways, were able to dispense with the usual monopolies by saying that competition is free. (There are at least two other privately held British airlines that could compete more effectively if allowed.) The Netherlands government is in a similar condition, eager that aviation should become a business rather than a branch of government.

The crucial step that may eventually unlock the jam is that the European Court has ruled that the Treaty of Rome, which created the "common market" in the first place, applies to aviation as to other kinds of business. At last, the Commission seems to have woken up. There is now talk that it will be writing to European airlines, possibly this week, with a warning that they must stop price-rigging and their other restrictive practices. The snag is that the Commission's only sanction is to take offending companies to the courts, not a speedy remedy. That is why hopes still centre on the prospect of a deal between the governments.

But what? The difficulty is that even the most fervent advocates of liberalized European air traffic seem to shrink from outright deregulation. Even the visionaries suppose that air traffic within the ten member states of the Community will continue to be regulated by a series of bilateral agreements between governments, so that aircraft bound from, say, Rome to London calling at Paris will not be free to pick up extra passengers on the second leg of their flight unless that right has

been separately negotiated. Instead, the hopefuls model their ambitions on the present agreements between the British government and those of The Netherlands and Belgium, which allow all airlines to fly between designated point at the frequencies they choose, and with whatever fares they fix, provided that both governments choose not to object. These arrangements are better than nothing, but fall short of what they might be. What Europe needs, in this connection as in others, is true competition. In particular, it should be understood that subsidizing airlines is as illegal as subsidizing, say, motor manufacturers (for, otherwise, wasteful carriers will be perpetuated), that airlines of any European nationality may fly between any pair of cities already connected by scheduled services and that there should be no restraint on the ownership, within Europe, of privately held airline companies. That is what the airline business will have to come to in the end. The European Commission should face up to that now, not later. Until it does, the benefits of a simple technology will remain in escrow. □

Broadcasting by numbers

Another British government has failed again to find a way of living without the BBC.

If we have enemies like that, what need have we of friends? That must be the reaction of the British Broadcasting Corporation (BBC) to last week's report of the committee under Professor Alan Peacock set up just over a year ago to find politically more palatable ways of supporting British public broadcasting, which is at present done by requiring those who use television receivers to buy an annual licence to make the practice legitimate. The Peacock committee came into being to spare the present government and its successors (of all parties) the periodic problem of deciding how much the licence fee should be, which is politically as popular as deciding by what degree politicians' salaries should be increased. The present government's suspicion (conviction?) a year ago was that advertising was the answer.

Expectations have not been realized. Peacock says that there is not enough advertising to sustain both public service broadcasting and the commercial stations. That is why the BBC is temporarily relieved. But then the committee goes off in a quite unexpected and technological direction, saying that the problem of financing the BBC independently of government decisions about a licence fee will be solved in the distant future, when it becomes possible to ensure that people pay each time they watch a television programme put out by the public broadcasting network. To the Peacock committee, it seems a minor matter that the technology for bringing this about, easily designed on the back of an envelope, is still far beyond most manufacturers' ken. It is nevertheless odd that a committee apparently willing to accept its marching orders, to devise alternative financing, should have sought refuge in technological futurology from the awkward truth that free-market remedies in present circumstances would kill standards. It would have been better to say outright that the BBC, for all its many faults, is probably worth keeping the way it is. □



Space vehicles

British HOTOL closes in on French Hermes

BRITAIN'S horizontal take-off and landing space plane, HOTOL, could be in space by 1998, according to the director of the British National Space Centre (BNSC), Roy Gibson. A bid to make it a European project will be made at the next (October) council meeting of the European Space Agency (ESA). HOTOL, which was little more than a gleam in a British Aerospace designer's eye a couple of years ago, thus seems to be racing ahead. With its launch date brought forward, what once seemed almost science fiction is now in direct conflict with the more conventional French project Hermes, in which a mini-shuttle could be launched on top of a cryogenic Ariane-5 rocket around 1995.

But will HOTOL work? According to British Aerospace, the design incorporates a "breakthrough" in air-breathing engine technology which makes it possible to move ahead with off-the-shelf technology rather than the completely new engineering needed for the US National Aeronautics and Space Administration's "National Aerospace plane" (NASP) programme. The NASP programme is aimed at pushing ahead with research in high temperature materials and computational hypersonic fluid dynamics to enable, early next century, the creation of a whole class of vehicles from long-range airliners to spacecraft capable of air-breathing propulsion at speeds of Mach 5–12. By comparison with HOTOL, NASP is "technology driven" according to the British Aerospace Launch Vehicles Project Manager, Bob Parkinson. "HOTOL is a conceptual, not a technological breakthrough", Parkinson claims.

According to Parkinson, "we saw a way of doing the propulsion system that no one else has spotted... We took the engine concept to Rolls-Royce" and HOTOL began to roll. That was a couple of years ago. But the "breakthrough" is still classified. Commentators have suggested the engine might precool inlet air with its liquid hydrogen fuel, to increase the density of the air and allow the production of a more compact engine. A Rolls-Royce spokesman said that the same engine, once the vehicle reached Mach 5, would be switched to liquid oxygen oxidant to complete the push into space.

Professor J.E. Ffowcs Williams, of the Department of Engineering at the University of Cambridge, many of whose staff work on Rolls-Royce contracts, said that though he knew little about the project he believed it would benefit from publicity at

this stage. Arthur C. Clarke, the science fiction writer, said from Colombo last week that he invented the idea of the air-breathing engine for space vehicles "40 years ago". But Clarke could not speculate on the British Aerospace design because "I don't know anything about engines and aerodynamics".

Meanwhile, Gibson and others backing the British space effort have the task of raising money from abroad for what is estimated as a \$4,500-million project to produce two flying HOTOL vehicles by 1998, without the freedom to expose the HOTOL "breakthrough". Nevertheless, says Gibson, BNSC has made "progress" in getting HOTOL declassified, as the classification was much more commercial than military. "We've been able to lift the veil enough to make a tour round Europe", seeking participants.

According to Gibson there remain a few technological "hoops", but all should be clear a year or so from now. The British plane is to present ESA members in October with a proposal for a four-year, \$400-million HOTOL feasibility study; but this will clash, politically at least, with the simultaneous effort being made by the French to win participants in a cheaper, \$48-million study of their Hermes space shuttle. Hermes is ahead on the political timetable, as ESA passed an "enabling resolution" approving the study in June (only the money is yet to be found); and it is also ahead on the engineering timetable (as the French space agency CNES has been working on Hermes far longer than the British on HOTOL). But Germany has been hesitating to give full support to Hermes, and according to a Bonn research ministry spokesman, HOTOL has already won the heart of his minister, Heinz Reisenhuber.

France has said it will go it alone with Hermes if it cannot raise European support. Britain, however, is sounding equally bullish about HOTOL. According to Gibson, whose first British space plan will go to ministers this week, participation of the French in the HOTOL programme would not be essential.

There is no French technology that can not be found or developed elsewhere, according to Gibson, and even French money may not be essential. It is not inconceivable, therefore, that HOTOL will go ahead like Concorde, the Anglo-French supersonic airliner, as a bi-national project, though this time the nations involved may be Britain and West Germany.

Robert Walgate

Indian science

Making the numbers count

Bangalore

THE gulf between the industrialized nations of both East and West and developing countries such as India is dramatically illustrated in the latest batch of statistics from the Indian Department of Science and Technology. In one year, India's spending on research and development amounts to just US\$1.52 per head of population, compared to \$361.22 in Switzerland, the country at the top of the league. The United States spends \$303.9, Sweden \$242.26 and Japan US\$231.1. With India at the lower end of the scale are the Philippines (\$1.65) and Pakistan (\$0.49).

The pattern is similar when research spending is seen as a percentage of gross national product (see table) or when the

Expenditure on research and development as percentage of gross national product (1982)

Sweden	4.9
Hungary	3.3
Czechoslovakia	3.8
United States	2.5
Japan	2.4
West Germany	2.4
India	0.85
Greece	0.2
Pakistan	0.2
Philippines	0.2

science enterprise is assessed in terms of the number of scientists and technologists per head of population. Compared with nearly 140 per thousand of population in the United States, for instance, India boasts a mere 2.63 per thousand. Yet in a country as big as India this means there were 2.47 million scientist and technologists in 1985, up from 1.95 million in 1980.

It is this two-million-plus science workforce that Prime Minister Mr Rajiv Gandhi sees as the key to India's welfare in the twenty-first century. His new science policy identifies three levels of projects: "missions", where scientists will need to put proven technology to work to solve specific problems; "thrust areas"; and "blue-sky" basic research. The emphasis in each area, is to be on the possible benefits for the "common man", most particularly India's rural population.

Five of the "missions" have already been selected — improving drinking water, campaigning against illiteracy, telecommunications, a vaccination programme for children and in agriculture a major effort to improve oil seeds. The "thrust areas" so far proposed are computer technology and biotechnology — frontier disciplines requiring major investment but with the potential to give immediate results.

Radhakrishna Rao

Scientific American

Unwanted fly in merger ointment

THE fate of 141-year-old *Scientific American*, which claims to be the oldest periodical in the United States, hung in the balance earlier this week, after an agreement by the board of Scientific American Inc. to merge with a West German publisher had been confused by a rival bid from Mr Robert Maxwell, the British publisher and owner of Pergamon Press. Mr Maxwell was bidding through the vehicle of the public company, BPPC, in which he holds a controlling interest. It will be for the shareholders in Scientific American Inc., which is not quoted on the stock exchanges, to decide whether to accept the board's proposal or that from Maxwell.

The board's proposal, agreed at a meeting in New York on 30 June, is that the West German company Verlagsgruppe Georg von Holtzbrinck of Stuttgart should be allowed to offer the shareholders of the US company \$258 for each of their shares, amounting to a total of \$52.6 million for the company as a whole.

Holtzbrinck's business centres on the publication of the newspaper *Handelsblatt*, a financial daily, but the company has been since 1981 a partner in the publication of the German edition of *Scientific American* (under the title *Spektrum*) and has recently been expanding into the US publishing industry by the acquisition of book-publishing companies.

Under the proposed arrangement, which includes the sale of the book publisher W.H. Freeman Inc., *Scientific American* would continue to operate as an independent enterprise, with Mr Gerard Piel continuing as chairman of the management board and his son, Mr Jonathan Piel, as editor and publisher. The younger Piel said last week that Holtzbrinck was enthusiastic about his plans for increasing the coverage of news in the magazine.

The present uncertainty is an ironic outcome of a process extending over the past three months, since the impending sale of Scientific American Inc. was first announced, and during which the New York investment (merchant) banker Salomon Brothers was commissioned by the board to conduct a "regulated auction" of the company. This is a process by means of which potential bidders can be screened so as to exclude unwanted owners. Sealed bids were received on 16 June from seven different organizations, including one from Maxwell of \$34 million.

Even the idea that *Scientific American* might be put on the block would have seemed strange a short while ago; during the 1960s, the monthly magazine was among the most successful of internationally circulating publications, both commercially and in reputation. In spite of its antiquity, *Scientific American's* success is

entirely a post-Second-World-War phenomenon deriving from the acquisition of the title by Gerard Piel and Denis Flanagan, the editor until four years ago.

The need to sell the company has largely arisen because of the discontent of some shareholders. The proportion of shares controlled by Gerard Piel is believed to be about one-sixth, not enough to prevent a sale. The largest shareholder, with 32 per cent, is Mr Arthur Seckler, well known for charitable works.

The company's announcement last week of the agreement with Holtzbrinck said that the board had confirmed this arrangement in spite of the receipt of a letter "from the bidder who had originally offered the lowest price... purporting to raise that offer to \$61 million".

Although the company says that it has legal grounds for not recommending Maxwell's bid to its shareholders, there is no obvious reason why the shareholders collectively should not let strictly financial considerations determine their decisions. The next step is, apparently, that Holtzbrinck will make a formal offer to buy the shares. A spokesman in Maxwell's office said this week that it would be for the board of *Scientific American* to consider the BPPC offer in the "light of its fiduciary

responsibilities to shareholders".

The uncertainty has nevertheless caused consternation among authors of W.H. Freeman, who appear to be alarmed at the transfer of ownership to Maxwell. Dr Harvey Lodish of the Massachusetts Institute of Technology says that he will not revise the first edition of his successfully published book if the threatened deal goes ahead. Dr Peter Atkins of the University of Oxford, whose successful textbook *Physical Chemistry* is published in the United States by W.H. Freeman, says that neither the shareholders nor the purchaser should be in doubt that the sale may be that of a "shell" if Freeman authors choose to defect.

Professor Martin Rees (Institute of Astronomy, University of Cambridge), who is writing a book for Freeman but who has not yet signed a contract, says that he is more offended by statements in a circular letter to BPPC shareholders three months ago explaining the basis which the titles of 361 titles of scientific journals were transferred from Pergamon Press to BPPC in exchange for £240 million. The circular says that, because scientists are more concerned with quality than price, prices of journals can (and have been) increased "well above the rate of inflation". In 1985, the prospectus says, the 361 journals will make a profit "before sales commission" of £23.7 million on a turnover of only £49 million.

John Maddox

Others have problems too

POPULAR science magazines have suffered serious falls in advertising revenue recently from which even the relatively serious *Scientific American* has not been excepted. According to the US Publishers' Information Bureau, *Scientific American's* advertising revenues have fallen by 27 per cent in the past year. The slump in the home computer market is one reason; although corporate advertising partly made up the shortfall, it too fell in the last year. Sales, at around 600,000 copies, are down from a peak of 720,000 in the late 1970s, mainly because of competition from the more popular magazines.

Mr Gerard Piel, chairman of Scientific American Inc., denies, however, that reduced revenues were the immediate cause of the sale; rather, he blames the "extremely turbulent corporate market" of recent years and the "staggering" frequency of mergers which threatened *Scientific American* with the possibility of a takeover by an unfriendly bidder who would not uphold the magazine's standards.

Falling advertising revenues are, however, blamed for the demise two weeks ago of *Science 86*, the popular publication of the American Association for the Advancement of Science. Time Inc. bought the magazine's assets for \$6 million and turned

the subscriber list over to its own popular magazine *Discover*, which can use the *Science 86* logo for 2 years. *Discover* has, however, been drifting away in recent months from science towards being a general consumer/ current affairs magazine. *Science 86's* advertising revenues dropped by 50 per cent in two years. Editorial staff are being "terminated".

Why has there been such a precipitous decline in advertising in popular science magazines? Different people have different answers, but the decline of the home computer market — and competition from specialized home computer magazines — is frequently mentioned. Some advertising agencies believe that readers of such magazines are not sufficiently consumer-minded, and that similarly affluent readers can be reached to greater effect in consumer magazines, which are also read by more people per copy. Others point to proliferation of popular science magazines in the early 1980s. But many agree that one more magazine will have to go before equilibrium is reached. The most vulnerable, by common consent, is *Science Digest*. Its advertising revenues fell by 50 per cent in the past year, and even the wealthy Hearst Corporation is unlikely to continue supporting a loser for ever. Tim Beardsley

AIDS

New centres for clinical trials

Washington

As the number of cases of acquired immune deficiency syndrome (AIDS) continues to snowball, finding an effective therapy becomes increasingly urgent. The US National Institute of Allergy and Infectious Diseases (NIAID) announced last week that it was committing \$100 million over the next five years to speed the search for a therapy.

Fourteen AIDS treatment evaluation units will be set up around the United States to test drugs that have shown some promise, either by blocking replication of the virus that causes AIDS, or by strengthening immune system function. Therapies likely to be tested in the first year of trials include the anti-viral compounds azidothymidine, foscarnet, HPA-23, ribavirin, interferon-alpha and possibly dideoxycytidine. In announcing the new units, Anthony Fauci, director of NIAID, said a successful treatment would probably combine an anti-viral drug with an immune modulator. But combination therapies will be tested only after each single agent is tested.

A Data Safety Monitoring Board will evaluate the progress at each centre recommending new directions on initial results. To streamline exchange of information among the 14 units, NIAID plans to award a contract this September for a trial coordinating centre.

While Fauci estimates that some 2,000 patients will be included in trials by the end of the first year of the programme, the centres are not intended for treatment of the nearly 22,000 people with AIDS in the United States. Plans for placebo-controlled clinical trials has renewed the debate over the ethical justification of such an approach. Robert Levine, chairman of the Yale University School of Medicine Institutional Review Board, has suggested that using historical controls may be an "ethically less problematic" research design than placebo controls.

But Walter Dowdle, AIDS coordinator

for the Department of Health and Human Services Public Health Service (PHS), says that controlled trials are the "most compassionate way to go". Dowdle argues that, in the long run, only placebo-controlled trials will show which therapies are truly effective. Dowdle is also sceptical about providing experimental drugs to patients not involved in clinical trials. As no agent has been shown to be safe and effective against the AIDS virus, providing unproven drugs to patients could accelerate the course of their disease.

Critics of the government's response to the AIDS problem say that the present

administration has not gone far enough in dealing with the problem. In an occasionally heated congressional hearing last week chaired by Representative Ted Weiss (Democrat, New York), Martin Hirsch of Harvard University testified that it is "a national tragedy that so few patients" are in controlled clinical trials for AIDS therapies. Dowdle and Fauci defended NIAID's plans, but when questioned, Fauci admitted that additional money would allow NIAID to support another five centres whose applications had received favourable reviews. Weiss was quick to insist that PHS should request the money in its 1987 budget. After the hearing, Dowdle said PHS was considering asking for more cash, but no decision had yet been made. **Joseph Palca**

OECD report

Sweden number one for research

SWEDEN "has more to teach than to learn" about research and technology policy, according to a team of distinguished examiners for the Organisation of Economic Cooperation and Development (OECD) whose report on Sweden has just been released*. Swedish industry spends more on research and development per unit of turnover than even Japanese industry, according to the report. Over the past 15 years, Swedish business has raised its receipts from patents sold abroad eightfold — while its payments for foreign technology licences has risen only fourfold.

Despite this success, the examiners complain, Sweden pays university professors only 50 per cent more than it pays university janitors. University posts remain unfilled and there is a danger that the education system will not continue to provide the technically trained manpower that Sweden's established high-technology industry needs. Between 1970 and 1984, the numbers of doctoral graduates in physics, chemistry and mathematics in Sweden fell 50–75 per cent. OECD lays the blame on a system of loans, repayable after graduation, to cover higher education fees. Loans discourage students from entering research careers. A further degree means further debts, and a doctorate does not lead to higher salaries, or at least not sufficiently high to pay off the extra debt.

Yet Sweden depends on science. Its pharmaceutical and biomedical industry spends 17–18 per cent of its turnover on research and development. Including government spending, Swedish spending per head of population on biomedical research is higher than that of the United States, the OECD examiners claim.

Swedish communications engineering, environmental research, energy-saving equipment, paper-making technologies and robotics are equally advanced. The

use of robots in Swedish industry had reached 29.9 per 10,000 workers by 1981 — the highest in the world, ahead of Japan (13.9 in the same year) and the United States (4.0).

The examiners congratulate Sweden on its enlightened workforce, and the "generally pro-science and technology attitude" in the country. This might be attributed, they suggest, to a strong programme by the national council for planning and coordination of research (FRN) in the schools, teaching children about energy problems, cancer, food and the like, an effort the examiners describe as "unprecedented in OECD countries with the possible exception of Japan".

The level of expenditure aimed at industrial development is now so high, however, that Sweden is in a class of its own, with no other country to learn from. Sweden is itself now an experiment, which other countries will watch with interest, to see the effects of this strategy.

A constant theme throughout this report from OECD, however, is that Sweden must beware of taking its renowned egalitarianism too far, at least in research. Swedish success in biomedicine, for example, is traced to the Nobel prize winner Theodor Svedborg and his successors at Uppsala; and the chemical industry to Nobel himself and precision instrumentation to Hasselblad. "We recommend that allocation of funds be made to individuals of excellence, with far-reaching plans, not to fields... and that high quality results be recognized by easy and substantial funding."

Robert Walgate

Centres named

The fourteen institutions receiving NIAID funds are: Harvard University; John Hopkins University; Memorial Sloan-Kettering Cancer Center; New York University; Stanford University; University of California, Los Angeles; University of California, San Diego; University of California, San Francisco; University of Miami; University of Pittsburgh; University of Rochester; University of Southern California; University of Texas M.D. Anderson Hospital and Tumor Institute; and University of Washington. □

*OECD Reviews of National Science and Technology Policies: Sweden (OECD Paris, 1986). The review panel consisted of: Guy Ourisson, director, Institut de Chimie des Substances Naturelles, Gif sur Yvette, France; Walter Zegveld, director, Policy Studies and Information Group, TNO, Delft, The Netherlands; Derek Denton, director, Howard Florey Institute, of Medicine, University of Melbourne, Australia; and Yrjö Toivola, managing director, Vaisala Oy, Helsinki, Finland.

Launch vehicles

France and US explain failures

AFTER third-stage ignition failures which destroyed two of the last three launches, the European space rocket Ariane looks set to go back to the makers for six months. The independent commission of inquiry which reported in Paris last week attributed Ariane's latest problems, and uncertain ignitions and failures early in the programme, to the small size of the "pyrotechnic" that lights the engine.

This view appears to counter speculation in the *Los Angeles Times* earlier this week that senior French sources believed Ariane had been sabotaged, but Jacqueline Schenkel of the Arianespace Washington office would say this week only that sabotage "had been practically ruled out". Certain questions are in fact "still being investigated" she said.

Ariane's third-stage, liquid hydrogen-oxygen engine is ignited by a four-second pyrotechnic burning in the combustion chamber. But sometimes the fuel has failed to ignite; at others it has back-fired and gone out or faltered but continued. Ignition has proved to be sensitive to too many factors. The tolerances of the process have to be broadened, the commission says, and the easy solution is a bigger pyrotechnic.

This will take approximately six months, but in the face of similar launch difficulties in the United States with the shuttle and conventional rockets, Ariane-space is still enjoying a burgeoning order book. Orders for 36 satellites to be placed in orbit at a net cost of some £1,000 million are still in hand, the company says.

Meanwhile, in Washington, explanations for US rocket failures abounded last week. At the Pentagon, the Air Force explained why its Titan 34D booster exploded seconds after lift-off. Over at the National Aeronautics and Space Administration (NASA), an investigation revealed faulty wiring as the cause of the failure of a Delta rocket on 3 May. Neither investigation showed fundamental flaws in rocket design, and launches could resume as soon as new prelaunch procedures can be implemented.

The Titan's problems started when rubber insulation apparently pulled away from the steel wall of the solid rocket motor (SRM) shortly after lift-off. This allowed combustion products to reach the booster wall, weakening it to the extent that it could no longer withstand the pressures inside the booster. Approximately 9 seconds after SRM ignition, the rocket exploded, causing \$70 million damage to the launch pad and adjacent facilities.

The Titan 34D SRMs are similar in design to those used on the space shuttle, but have a different manufacturer — United Technologies rather than Morton Thio-

kol. The commission investigating last January's shuttle disaster concluded that a failure of the O-ring seals caused the Challenger accident. But General Nathan Lindsay, who headed the Air Force investigation, said the O-ring seals joining the solid rocket segments "did not contribute to the [Titan 34D] mishap".

Lindsay does not believe that the accident represents a flaw in the SRM design. He pointed out that 940 booster segments have launched successfully without a problem. The Air Force plans to upgrade its inspection procedures of the solid rocket motor segments, but otherwise is

making no major alterations in the Titan 34D as a result of the accident. Lindsay expects that Titan 34D flights will resume within a year.

The problems of the Delta rocket were primarily electrical, according to NASA's investigation. A short in the main engine control circuits caused both the main and altitude engines to shut down. Without controlling thrust from the engines, the Delta broke apart in the atmosphere and was destroyed by ground controllers. Vibration at lift-off apparently caused the erosion of some insulation in a wiring harness, leading to the short circuit. NASA's investigation board recommended redesign of parts of a wiring harness, as well as increased attention to detail in construction. **Robert Walgate & Joseph Palca**

Nature conservation

Too many of the wrong trees?

IN a country as stripped bare of woodland as Britain, it might seem certain that the planting of new forests would be universally welcomed. But last week, all the nation's major voluntary conservation bodies



joined hands in a report protesting against the rapid growth of Britain's forests.

The report comes from Wildlife Link, which represents 24 conservation organizations with a total membership of 1.4 million. It is not more trees that they find objectionable but that present government policy, grants and tax relief favour a type of forest practice which they believe is damaging to the environment. The pace of planting has certainly increased: nearly 5.5 million acres of new forest a year, double that of fifty years ago. And, says the report, much of that is in massive monocultures, usually of Sitka spruce, that are transforming open land habitats and destroying the flora and fauna of moorland, heath, bog and sand dune. Two and a half million acres have gone since 1919. Vast areas of Scotland are now "tree farms" which destroy wildlife and are utterly different from any native forest that might once have grown there.

The critique by the conservation organizations comes a week after the publi-

cation by the Nature Conservancy Council of *Nature conservation and afforestation in Britain*, which details the increasing conflict between forestry interests and conservation needs. Each of the arguments for further massive plantations — that there is still plenty of open land left; that, as much of what is now open land was once forest, afforestation is a return to a more natural environment; and that forests support more wildlife than open ground — is examined and found wanting. Nor does the report find economic arguments compelling. Although Britain imports 90 per cent of its timber so that the need for increased domestic production would seem vital, forestry gives a low return on capital invested and takes up funds that might be better spent elsewhere.

The conservation bodies would like to see more sensitive planting, particularly using native tree species, in a manner that would help to increase the diversity of wildlife and avoid damage to unique open habitats. Various possible new planning control methods are suggested, but the first task remains to notify all Sites of Special Scientific Interest (SSSI) and to ensure that they are adequately protected. One possibility might be a stronger regulatory role for the Forestry Commission which processes applications for forestry grants. But the commission would first have to strike a better balance between timber production and protection of the environment in its own activities.

In the longer run, new and more attractive forestry schemes may appear. There is pressure within the European Community to reduce excess food production by converting relatively fertile farmland to forests. These lowland forests could be of native hardwoods and support rich wildlife communities; quite different from the massive forests being grown on the north-western moorlands. **Alun Anderson**

National Science Foundation

Grant applications go paperless

Washington

EXPRES, the National Science Foundation (NSF)'s plan to make grant application all electronic, will take off at the end of this month. NSF hopes the project will streamline its review process and serve as a prototype for the exchange of scientific information between different computer environments. But success depends largely on factors outside NSF's control.

Formally titled Experimental Research in Electronic Submission, EXPRES

Eucalyptus clean-up

New Delhi

INDIA'S northern state of Haryana appears to have found one solution for a major problem of developing countries — how to dispose of sewage cheaply. A five-year project at the Central Soil and Salinity Research Institute (CSSRI) at Karnal has revealed that untreated sewage is just the thing to encourage eucalyptus forests to grow on wasteland.

When the CSSRI scientists began their project back in 1981, they simply pumped sewage into trenches on wasteland and planted eucalyptus trees on ridges between them. Now the wastelands have turned into eucalyptus forests, previously used sewage ponds have disappeared and there are fewer mosquitoes.

"Land disposal of sewage is the cheapest and most cost-effective method of disposing of urban waste waters as it utilizes the entire biosystem — soil and vegetation — as a living filter", says Dr Ranbir Chhabra, a senior soil scientist at CSSRI. Untreated sewage contains large quantities of plant nutrients but the presence of pathogens, heavy metals, pesticides and above all its odour makes it unsuitable for growing agricultural crops and vegetables. But these considerations do not affect its use for growing trees which are used as timber and fuel but not as food, says Chhabra. As the trees act as biopumps, they prevent water-logging and thus groundwater pollution.

There are two reasons why the eucalyptus system is particularly attractive to the Haryana towns. First, CSSRI has shown that one hectare of eucalyptus irrigated by sewage water can earn the municipalities a gross return of Rs 9,700 (£600) in the first year and Rs 28,000 (£1,800) in the second year. The sludge accumulating in the furrows, which can be sold as manure, is a bonus. Second, the technology raises the prospects of developing plantations into green belts and scenic parks. No wonder the project has the approval of the state forest department as well as the pollution control board.

K.S. Jayaraman

would shift proposal development, submission and review from reams of paper to the realm of computer workstations, so that a proposal could be distributed from its origin to NSF, and from NSF to reviewers, entirely by electronic transmission. Because the grant-seeking community is both technologically and geographically diverse, NSF thinks the scheme makes the perfect guinea pig for research on information exchange. The foundation receives ten copies each of 37,000 proposals from about 2,400 organizations in an average year.

The inspiration for EXPRES came from the NSF office that processes grant proposals, and gained impetus when Erich Bloch, a former vice-president at IBM, became NSF director in 1984. The foundation expects to announce two award recipients in September, and has earmarked \$2 million for EXPRES in fiscal year 1987.

Although the duration of the awards is three years, funding for the remaining two is not "set in concrete". Competition for the awards may be keen nonetheless: an interest meeting last month drew more than 30 people, and nearly 200 organizations have requested project solicitations.

Part of the reason that EXPRES has sparked this response has to do with its applicability beyond the paper-shuffling procedure at NSF; as project manager Alvin Thaler claims, "the grant process is just a handle to grab hold of the whole problem of communicating scientific information". Ultimately, however, the project's implementation depends upon concurrent developments in computer standardization to lay the groundwork for open information exchange. EXPRES pilot installations will initially use NSFNET, a network that links the foundation's supercomputers and is itself still in its formative years. Although NSFNET currently conforms to DARPA protocol (a system developed by the Department of Defense), it will eventually adopt the universal Open Systems Interconnection (OSI) standards of the International Standards Organization. But OSI standards are also in their infancy, putting another question mark in the EXPRES timetable.

The birth of EXPRES, therefore, needs to be closely attended by the groups responsible for computer standards, such as the National Bureau of Standards and the American National Standards Institute.

The project may also have an ally in the Virginia-based Corporation for Open Systems (COS), an organization conceived just last year to accelerate implementation of OSI by coordinating industry efforts towards standardization. With 55 members, including IBM, AT&T and Digital Equipment Corporation, COS could

hasten the industry-wide compliance that NSF needs to make EXPRES operable. The view from COS is clear: programme manager Vance Mall says that if all concerned parties pool their efforts, EXPRES could be up and running by 1990.

Karen Wright

Vane back to basics

WITH the financial backing of Glaxo, the British pharmaceutical company, Sir John Vane is going back to his pharmacological roots, a year after leaving the Wellcome Foundation, of which he was research director.

Vane will direct the new William Harvey Research Institute at St Bartholomew's Hospital Medical College, situated close to



St Bartholomew's Hospital, where William Harvey established that blood circulates, but even closer to Smithfield, London's central meat market. Funds will be available for about a dozen scientists plus support staff for at least five years to carry out research on cardiovascular disease, with particular emphasis on atherosclerosis and eicosanoids, the area of research for which Sir John shared the 1982 Nobel prize for Physiology or Medicine.

The first of Vane's priorities is to stock the institute with Watanabe rabbits or other animal models of human atherosclerosis. With these and with the aid of cell culture and his own cascade bioassay system, Vane intends to improve the understanding of the pharmacology of cardiovascular disease. Against the trend, he has no plan to use the techniques of molecular genetics in the institute's research.

In return for financing the new institute, Glaxo has first rights on any discovery of commercial interest to it. Sir John is the second top Wellcome researcher to be snapped up by Glaxo; earlier this year, Dr Pedro Cuatrecasas left his position as head of research at Burroughs Wellcome, the US subsidiary of Wellcome plc, to set up a US research operation for Glaxo Ltd.

Peter Newmark

Animal welfare

Laboratory animals unprotected

Tokyo

DEBATE over the welfare of laboratory animals in Japan has been sparked by reports from a French scientist that monkeys at Kyoto University's Inuyama Primate Research Institute have been shamefully maltreated. Bernadette Brésard, who carried out research at the institute last year, has revealed to French magazines that monkeys were routinely restrained in "monkey chairs" for months on end. Behind this one incident lies a much deeper problem: the lack of clear, enforceable regulations on the treatment of laboratory animals in Japan.

Brésard came to Japan last year with an

orang utan, Dou-dou, and a chimpanzee, Chloé, which were exchanged for Japanese macaques from Inuyama. The Inuyama centre, funded by the Ministry of Education, Science and Culture (MESC), was set up as the nation's key primate research centre. But after being recruited by MESC to continue her research on cognitive ethology Brésard soon found herself embroiled in an argument.

The monkeys were confined day and night for several months in makeshift chairs with a stock-like clasper device around their necks so that electrical recordings could easily be taken from their brains. The monkeys' necks became ulcerated and their testicles degenerated because of the prolonged immobilization.

Horried by the monkeys' condition, Brésard requested their immediate release, but she was told by members of the psychology department to which she belonged that they had "no right to ask the researchers to release their monkeys as long as they considered it essential for their experiments". Instead, Brésard was nominated to join an informal working group on animal welfare set up by associate professor Toshio Asano of the psychology department who also chaired the institute's *ad hoc* animal welfare committee. But, frustrated by the working group's prolonged deliberations, Brésard turned to the French magazines, *Geo* and *La vie des bêtes*.

No sooner did the articles appear than the Japanese embassies in France and Switzerland were inundated with complaints and the Foreign Ministry asked MESC to investigate. But the Inuyama researchers pointed out that they had been acting within Japanese law and government guidelines.

Whereas Western nations have been tightening up guidelines and introducing new laws, Japan still has regulations that are full of loopholes and leave interpretation largely to the "common sense" of the researcher. For example, in Article 5 of the guidelines issued by the Prime Minister's office in 1980, researchers are told to "minimize the pain and suffering of laboratory animals by, for example, giving anaesthetics", "provided this does not interfere with the purpose of the experiment".

The regulations have no teeth. There is no system of licensing or government inspection to check that researchers follow the guidelines, no process of review of research proposals, and no requirement to report the number or type of animals used or the experiments carried out. Hence the fine of up to 30,000 yen (£120) for cruel treatment has little chance of being levied.

There are no vociferous anti-vivisectionist groups in Japan campaigning for

animal rights, as there are in the West. The Japan Animal Welfare Society is a moderate group that seeks to reduce experimentation on animals and to alleviate their suffering.

Recognizing that Japan lags behind in animal welfare, the Inuyama *ad hoc* committee headed by Asano drew up guidelines for the care and use of laboratory primates based on the US National Institutes of Health 1985 guide. Published in April shortly after Brésard left, the Inuyama guide is backed up by an institutional monitoring committee composed of faculty members, including at least one

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Courtesy of A. Okada/Newton.

who is involved in animal experiments; one who is not; and a veterinarian.

With implementation of the guide, conditions for the restrained monkeys have been considerably improved at Inuyama, says Asano. Chairing beyond 24 hours is no longer permitted without consent from the monkey committee, and no requests for chairing beyond 24 hours have so far been made. When the monkeys are not being used for experiments, attempts are made to bring them together in social groups. Minimum cage sizes are defined and, although cages at Inuyama do not all come up to standard, Asano says they will soon get the 300 cages they require, despite a price of about 150,000 yen (£600) per cage.

The Inuyama guide is now attracting attention. At the annual convention of the Primate Society of Japan in Nagoya in mid-June, members were urged to draw up their own guidelines following that of Inuyama. But the Inuyama guide avoids discussion of an issue that has dominated debate in the West; pain in laboratory animals.

A symposium on this topic was held in Tokyo at the end of May by the Laboratory Animal Society of Japan, but the participants tied themselves up in semantic knots and no significant progress was made.

Although changes have now been made at Inuyama, other institutes are under no obligation to adopt new guidelines. Nor is there any way of checking up what conditions are like in private industry, where there is no requirement to report experiments. Changes in legislation are not at present being considered by the government, but there may be changes in attitude if concerned groups continue to grow in strength.

David Swinbanks

Eiffel in orbit

FRENCH engineers, clearly inspired by the recent refurbishment and laser-lighting of the French-built Statue of Liberty in New York, are now planning to put an Eiffel Tower in space.

The object of a competition, announced last month by the Eiffel Tower Company (Société Nouvelle d'Exploitation de la Tour Eiffel), is to design a structure for the space age "as audacious and imaginative as that of Gustave Eiffel for the age of steam". Designs must be for a durable structure visible to the naked eye and symbolizing

Perhaps a little too fanciful...



"universal communication, the great dream of our times". Otherwise, the structure is to be useless: it must have "no commercial or military function", but "peaceful" and "scientific" uses can be considered. The three best projects will be selected by a jury of scientist, space engineers and artists this October, for prizes of up to \$20,000. There will also be a junior category for under-15s. Actual construction of the winners' projects, however, is not guaranteed.... Further information may be obtained from: La Tour Eiffel de l'Espace, Champ de Mars, 75007 Paris, France.

Robert Walgate

US nutritional research

Your grant is what you eat

Washington

NUTRITIONISTS find themselves divided on the activities of United Sciences of America (USA) Inc. Founded just six months ago, USA intends to put some \$1.2 million a year into nutritional research; the trouble is that the company's profits come from "network marketing" of "revolutionary dietary supplements". Despite USA's distinguished scientific board, many experts are sceptical about the value of dietary supplements for the general population and object to USA's advertising.

USA was founded last year by Robert M. Adler, a telecommunications entrepreneur. Its promotional videos make much of the claimed scientific merit of USA's preparations; a slickly produced 1985 video features views of huge computer rooms where research data is stored, and brief shots of the covers of *Nature* and the *New England Journal of Medicine*. The scientific advisory board includes two Nobel prizewinners and other well-known medical researchers. Critics allege, however, that not enough is known about some of the ingredients to recommend dietary supplements.

In addition to a retainer (at least for some members), members of the scientific advisory board get first shot at USA's research awards. Two weeks ago, at a meeting at its Dallas headquarters, USA approved its first twelve awards totalling \$600,000, of which several went to board members. But the meeting also spawned disagreement among the advisory board on the company's use of members' names for endorsements. One member, Dr Alexander Leaf of Harvard Medical College, threatened to resign unless his name was removed from endorsements.

According to Dr Jeffrey Fisher, USA vice-president for product development, the company will eventually offer a nutritional plan, a weight management plan and an aerobic fitness plan. The catchword is "optimal health". So far only the nutritional plan is on the market: it consists of a preparation of vitamins, trace elements and amino acids billed as rich in anti-oxidants; a "fibre energy bar" that includes a complex mixture of carbohydrates and a proprietary blend of cotton seed and surimi protein; a calorie control formula, again with special proteins; and a marine lipid concentrate containing omega-3 fatty acids, antioxidants and garlic extract.

Many of the ingredients are the subject of legitimate research. But many nutritionists question the strength of the evidence on which USA's products are based. Some, such as Dr Robert Good of the University of Southern Florida, chairman of the scientific advisory board, be-

lieve that indirect evidence is sufficient to recommend that the public take, for example, fish oils. But others, such as Henry Kamin of Duke University, who recently chaired a controversial National Research Council dietary study, believe that to offer fish oil supplements to the general public is "grossly premature".

Kamin — and many others — believes that epidemiological evidence (such as the low rate of heart disease among eskimos who eat a lot of fish) should not be used as a basis to "stuff people with fish oils" until possible hazards of long-term use are evaluated. Even if they could benefit some individuals, nobody has any idea of what would be a good dosage of omega fatty acids, according to Kamin. One of USA's recent research awards was to Leaf to investigate the effects of different levels of fish-oil supplements on blood chemistry.

Kamin also argues that there is no need for special protein blends with good amino-acid balance when the typical US citizen daily eats more than three times the amount necessary. He questions the relevance of the scientific advisory board's expertise; although a celebrity cast, few if any members are primarily nutritionists, a point admitted by Fisher.

Other features of the USA products are also contentious. Victor Herbert of Mount Sinai School of Medicine says there is "no evidence" that taking supplementary doses of vitamins E and C above national recommended daily allowances has any protective effect against cancer, a conclusion reached by the National Academy of Sciences in 1982.

USA's advertising is careful not to make any claims about specific health benefits, which would in the eyes of the Food and Drug Administration make the product a drug needing trials for safety and efficacy. But the 1985 video, which dwells darkly on environmental pollution and blames it for high heart disease and cancer rates, manages to suggest directly that USA's products might counter these hazards.

Leaf, chairman of the department of preventative medicine and clinical epidemiology at Harvard Medical College and a member of the scientific advisory board, is quoted in USA promotional materials as saying that "the four formulas of USA Inc. together constitute a nutritional plan that is the finest and most complete I have ever seen". But Leaf denies making such a statement and says he thought he had been "a little naive" in his dealings with USA Inc., having been told only that he would serve as a consultant. Leaf said he was threatening to resign from the advisory board unless his name was removed from product endorsements

and that other members had made similar protests. The American Heart Association's logo is also apparently used in the video without permission.

USA's sales method is a source of concern to some. Described by Fisher as "as innovative as the programs", it involves individuals becoming "associates" of the company and then recruiting more associates — similar to "pyramid selling". The Food and Drug Administration points out that it is difficult to police claims made for products sold in private. But Dr Robert Morin of the University of California at Los Angeles, another member of the advisory board, contends that the instructional materials provided by USA Inc. make "network selling" preferable to simple counter sales.

Good says the dietary programme has been established "within conventional scientific wisdom" to provide "under-nutrition without malnutrition". Good admitted that he had not seen USA's promotional video. But he is a believer in the benefits of anti-oxidants, which may inhibit some ageing processes. Good says he has been able to forestall death of genetically short-lived animals in all species so far studied, and believes there are sufficient data to promulgate cautiously dietary supplements to the public. And Good believes that a weight-reduction plan must include protein supplements.

Fisher admits that early videos were "too hypish" and says later versions were toned down. And he admits that there is debate about some nutrients in USA products but says science is coming around to USA's point of view. **Tim Beardsley**

Young Hungarian

SZEGED University's Department of Natural Sciences has called on the Hungarian government to cut military spending and uneconomic large-scale projects and to devote the money saved to improving higher education and the standard of living of young graduates. Although such issues have been raised before in Hungary's underground press, this is the first time that they have been explicitly stated in an official context, at least since the discussions that led up to the 1956 uprising.

The Szeged group, which consists of young academic and graduating students, submitted its proposals to the Congress of the Hungarian young communist movement in May. Officially they were ignored and, according to some unconfirmed reports, copies of the university newspaper, *Szegedi Egyetem*, which carried the proposals, have been impounded.

Even apart from the proposal that military spending should be "rationalized", the Szeged document is bound to be controversial. One proposal is that the univer-

Information technology

Where next for Alvey?

BRITAIN'S Alvey programme, aimed at strengthening the information technology industries through collaborative research with the universities, appears to be at a critical stage. Last week's Alvey conference made clear that the programme, now halfway through its five-year life, has produced enough successes to excite government, universities and industry. But nobody is sure how to keep the momentum going to ensure long-term industrial success.

The 600 delegates at the conference at the University of Sussex were clearly optimistic about the future. The Alvey programme is a unique attempt to break down the barriers between the universities and industry in key areas of information technology: very-large-scale integrated circuits, software engineering; intelligent knowledge-based systems; and man/machine interfaces. To ensure industrial involvement, even the smaller basic research projects carried out at universities must have an industrial "uncle" — someone from industry who will alert colleagues when an idea begins to look worth industrial development.

Three hundred and three projects are now under way at a cost of almost £200 million to the government plus a somewhat smaller amount from industry. One hundred and eight companies are participating alongside 53 universities and polytechnics (Imperial College, Edinburgh and Cambridge form the first league), a

academics protest

sities be given real, not merely symbolic, control over their budgets and the right to choose their own officials, rather than have them appointed by the state.

A further suggestion is that student grants should be pegged to keep pace with inflation and that less weight should go to means testing of family income. This would favour better-off families and is liable to be opposed by Party hard-liners.

Another proposal raises the whole question of the relationship between workers and intellectuals in a socialist state. In Hungary, entitlements to annual leave, pension rights and priority for housing are all related to length of service at a particular enterprise. Young graduates entering the system thus find themselves disadvantaged because they are five years behind in acquiring benefits compared with a worker who entered employment direct from school. The Szeged group is now asking for the years of study to be counted in the employment record — a seemingly modest proposal but one with clear political overtones.

Vera Rich

level of cooperation that Minister of State for Information Technology Geoffrey Pattie described as "probably unprecedented in peacetime". But as John Alvey, who originally proposed the programme, put it, "we have won some victories, but haven't won the war". The test will be the international marketplace. If saleable products cannot be produced, then Britain's industrial position will fall back still further.

Much discussion centred on what must be done to prevent this happening. Those actively involved in research tended to concentrate on the details: is there not, for example, an easier way to cope with drawing up cooperative agreements? Big delays have been caused. And would it not be wiser to have a central research institute instead of distributing work throughout the participating laboratories? Advantages of the former are that it quickly creates a critical mass of expertise and avoids duplication. But it can too easily become an ivory tower. The really big questions, spelled out by Pattie, were of government support and the balance between national and international programmes. Although everyone would like funding to continue, the pre-competitive research of Alvey must be commercially exploited and the postgraduates who have been trained persuaded to move on to industry.

For the balance between international and national funding, critical decisions must be made soon. The huge industrial high-technology programmes, ESPRIT and RACE (for information and communication technology), lie within the European "research and development framework programme", designed to shape policy for the next five years. The outline of the programme is now before the European Community's science ministers. Where will the post-Alvey programme fit? According to Pattie, international programmes cannot substitute for national ones, for the latter are necessary to give strength in collaborative projects.

A formal "After Alvey" committee, chaired by Sir Austin Bide, has already been set up and will report in the autumn. Submit your evidence to Sir Austin, said Pattie: "no decision has been predetermined" and "real decisions" will be made. But as one iconoclast at the meeting put it, "can any number of collaborative programmes be a substitute for the rationalization of Europe's information technology industries?" Without markets and companies of the size found in United States and Japan, can Europe's excellent research ever be successfully exploited?

Alun Anderson

Chinese science

Legal protection for scientists

CHINA is to have a new law to protect societies from "outside interference". Addressing the third national congress of the Chinese Association for Science and Technology (CAST) last month, Song Jian, minister in charge of the State Science and Technology Commission, noted that the proposed law would give "official status to scholarly societies and define their role". It would regulate their relationship with the Communist Party, the government and state-owned collective and individual enterprises, Song said, as well as guaranteeing that their work could proceed "along normal lines".

China has 138 learned societies and organizations with 1.8 million members, who, in their turn, according to Song, are "in touch with a further five million scientists and technicians". The need for legal protection for scientific and learned research has been much discussed in the Chinese media during the past two months, in connection with the thirtieth anniversary of the launching of the "double hundred" policy ("Let a hundred flowers bloom, let a hundred schools of thought contend") which in recent years has been restored to official favour. The Constitution of the Peoples' Republic of China (Article 47) stipulates that "citizens have freedom to engage in scientific research, literary and artistic creation and other cultural activities", but this did not save scientists and scholars during the cultural revolution. Promulgating a specific law to cover learned activities, however, is felt to be sufficient, in the words of the *People's Daily*, to "ensure the implementation of the 'double hundred' without any intervention by or hindrance from either 'leftist' or 'rightist' tendencies".

The new law, and the "double hundred" policy itself will not give the scholars total independence. Social scientists, economists and philosophers will be allowed, within their learned environments, to investigate and consider non-Marxist theories. If they are asked to provide recommendations for practical implementation in production or society, however, they must base their proposals on sound Marxist principles. The natural scientists come off somewhat better, since, according to the *Peoples' Daily* and other leading commentators, the natural sciences can only flourish properly when kept clear of misleading ideological and philosophical labels. Frequent reference has been made recently to the Qingdao genetics forum of August 1956, which broke the monopoly of Lysenkoism in China as the exemplar of the proper "contention" of scientific ideas.

Vera Rich

Why not move RGO to Manchester?

SIR—Alun Anderson's News article (*Nature* 321, 799; 1986) on the move of the Royal Greenwich Observatory (RGO) neglected to report that the Science and Engineering Council (SERC) also considered the possibility of moving it to Manchester. In fact, Manchester presented a powerful case, so why will RGO not move here?

SERC found the merits of Cambridge and Manchester to be finely balanced but simply considered the Cambridge case "to be even stronger". A world-beating centre of excellence is clearly what it was seeking, but that should not automatically favour Cambridge. The recent review by the University Grants Committee (UGC) rated Manchester's Physics and Astronomy Department, including Jodrell Bank, as outstanding, and here RGO would have formed the nucleus of a centre every bit as excellent as that we can expect in Cambridge. Did a beleaguered regional university just lose to the charisma of the old establishment?

An important inconsistency in the council's reasoning is that while claiming to be planning for the 1990s, it has allowed itself to be unduly influenced by the short-term consideration of minimizing disruption. Moreover, it does not give any reasons why the disruption caused by a move to Manchester should exceed that of a move to Cambridge. Could this be prejudice by the staff of RGO itself?

Alun Anderson's worries about costs are very relevant, but here Manchester had a strong advantage which was ignored. Construction of a new building to house RGO in Cambridge was apparently considered no more expensive than minor internal alterations to the suitable vacant premises in Manchester.

The council blithely asserts that both Manchester and Cambridge "have excellent communications" — sweeping Manchester's overwhelming superiority in this regard under the rug. The RGO staff will have to make regular trips to the new observatory in the Canary Islands; Manchester's international airport offers frequent direct flights and is just 20 minutes' drive from the university, whereas Cambridge to Gatwick airport is a long and awkward journey. It will be many years before Stansted will offer a service as good as already exists from Manchester. In addition, Manchester's rail and motorway connections to the rest of the country are far superior, which, together with its more central geographical location, would make RGO here much more readily accessible to astronomers around the country.

But the most disappointing aspect in my view is the missed opportunity for some positive action to redress the north/south

economic imbalance. The number of new jobs at stake is rather small, but the relocation of an influential and sophisticated scientific research organization to the north-west would be a morale-boosting statement of confidence in northern science and industry. Its demands for local specialized high technology, computer software and so on would be of much greater industrial benefit here than in the already thriving area around Cambridge.

We may never know what clinched the decision in favour of the old university and the south. But we should be told because it caused extra expense and could be to the lasting detriment of British astronomy outside Cambridge.

JERRY SELLWOOD

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Prehistoric protection

SIR—The letter by Fernandez and others¹ on radon in the Altamira cave shows how lax the standards of radiation safety were in Europe before 10,000 BC. Assuming that they worked a 40-hour week, I estimate that the palaeolithic painters received an annual radiation dose of 130 mSv from radon daughters. This would not be permitted under the Euratom legislation to which the European Community is now subject²: the dose limit for workers is 50 mSv in a year and indeed all exposures must be kept as low as reasonably achievable.

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1. Fernández, P.L. *et al.* *Nature* 321, 586–588 (1986).
2. Official Journal of the European Communities, No. L246/1, 17 September 1980.

Speaking up

SIR—David Swinbanks (*Nature* 321, 374; 1986) seems to doubt the relevance of the speech I made when accepting the Japan Prize.

An example of scientists not speaking up in time was the destruction by a Christian mob, around AD 415, of the famous library in Alexandria. That event set back civilization 1,000 years.

If third-century scientists had been able to enlighten the public, and if the government and the clergy had spoken out loudly and clearly at every opportunity, that set back would not have occurred. If we do not speak up now, our civilization may be destroyed for more than 1,000 years.

While in Japan, I learned that the United States is expected to provide a great deal of money for research for the

Strategic Defense Initiative (SDI) by West Germany (25 per cent), Great Britain (18 per cent) and Japan (13 per cent). The preliminary budget is \$60,000 million simply for research; development is not mentioned. You cannot blame the British Prime Minister for not refusing a subsidy to the British economy of 18 per cent of \$60,000 million.

President Dwight Eisenhower warned against the military-industrial complex, which has a momentum of its own. It has now extended far beyond the United States.

I also said in Japan that President Reagan has a better chance of achieving a nuclear freeze and a reduction of the nuclear arms race than any previous president. He should be told what scientists feel.

W.J. KOLFF

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Screwworm fly

SIR—In the past twenty years, despite one or two minor setbacks, the New World screwworm fly, *Cochliomya hominivorax*, has been eliminated from the southern United States and all but the most southern areas of Mexico. It is difficult to accept that this is due, as suggested by J.L. Readshaw (*Nature* 320, 407; 1986), solely to climatic conditions adverse to the survival of the fly during this period. It is surely more than a coincidence that this occurred concurrently with the major sterile insect release method eradication campaign in the region.

This campaign, by the US Department of Agriculture, has apparently eliminated this major agricultural pest, which caused losses of many million dollars to cattle ranchers in the southern United States and Mexico.

A related species, *Chrysomya bezziana*, widespread in South-East Asia and Papua New Guinea, is one of the most serious exotic animal disease threats to Australia. The United States has been most generous in sharing sterile insect release technology. This has enabled Australia to develop methods which it is hoped will be effective in eradicating any incursion of *Chrysomya bezziana* into this country. Whatever the beneficial effects of climate to the North American programme, the tropical climate in northern Australia has convinced us that rapid application of the US technology would be our major defence response.

R.W. GEE

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Anti-darwinism in Japan

SIR—L.B. Halstead's article on the so-called Imanishi phenomenon, the popularity of the writings of Kinji Imanishi, emeritus professor of Kyoto University, among intelligent laymen in Japan, attempts to relate the phenomenon to the character of Japanese society and its scientific community. Imanishi points out that the competition and selection in Darwin's view of nature derive from the cultural and religious ethos of Western paternalistic society, whereas the Japanese view is maternal, one that sees the inherently harmonious nature of the living world.

After holding a visiting professorship at the department of geology and mineralogy of Kyoto University, Halstead explained the Imanishi phenomenon by pointing out that the present world, Japan in particular, is demonstrably a world of competition, struggle and disharmony. In such a world or society, Halstead writes, "Imanishi's theory can satisfy the dreams and aspirations of people in a desperate rat-race, serving as a dream-scape sentimentality. Unhappily it has no place in scientific understanding of the real world."

Halstead's observation is rational, axiomatic and persuasive. Yet it creates a certain disharmony in the minds of "average laymen" in Japan. It reminds us of Niels Bohr, who extended his complementarity principle in quantum mechanics to the realm of human concerns beyond natural science. He states that the rational scientific approach is only one way of dealing with the world around us. A given approach seems fragile and senseless when applied within the framework (cultural tradition) of another, but is forceful and convincing within its own frame¹.

One of the central concepts of Imanishi's evolution theory, the principle of life-style partitioning which later evolved into the idea of the harmonious nature of the living world, arose out of his early research on aquatic insect larvae, mayflies in particular, in freshwater streams. On the basis of recent researches in freshwater, marine and terrestrial habitats in experimental ecology which demonstrated that interspecific competition takes place in 90 per cent of all cases studied, Halstead claims that the statistical foundation of Imanishi's theory no longer stands. He adds: "this seems to have little effect on either the standing or on the public reception of Imanishi's ideas so long as he states he is not an ecologist, since then ecologists cease to discuss his theories. When Imanishi further states he is not a scientist, fellow scientists no longer feel there is any need to discuss his ideas." Thus Halstead concludes his article with the statement that vigorous

debate and scientific confrontation are not part of the cultural tradition in Japan "... originality and innovation flourish in secret enclaves — Kyoto University saloon and coffee houses — beyond the experience of the ordinary Japanese, condemned, as they are, to the rigid authoritarian feudal society that masquerades as one of the advanced nations of the world."

My understanding is different. When Imanishi states he is not an ecologist, he is warning with A.N. Whitehead² that the narrowness in the selection of evidence in science is the chief danger to the construction of a holistic world view. When Imanishi states he is not a scientist, he is proposing that the ultimate concern and responsibility of a scientist should be to free contemporary intelligent laymen from their cultural fragmentation by making them more conscious of the way in which art, morality, religion and science have become specialized, censorial, constrictive to the unbroken wholeness of our cultural experience.

As Halstead points out, the fundamental divide in man's view of the evolution of life has been the degree of emphasis placed either on observed variations among individuals or on the features held in common. Then the harmonious nature of the living world exemplified by the phenomenon of altruism and habitat segregation becomes susceptible to an explanation in terms of natural selection. It would then be a responsibility of a careful scientist to try to replace words such as competition and selection by more objective terms or words that do not change the essential scientific tenets of darwinism but greatly change their aesthetic, moral and religious connotations. Without a critical usage of the words, the notion of the survival of the fittest, for example, would become a tautology of the selection of the selected or the survival of survivors³.

It is encouraging that the progress of molecular genetics over recent years has developed new concepts such as evolution by gene multiplication⁴ and concerted or coincidental evolution⁵ which are not inconsistent with Imanishi's ideal of the evolution of the harmonized holospecies in which all the individuals of a species evolve simultaneously when the time becomes ripe.

Needless to say, we are only beginning to understand the origin and the evolution of life. At present, we do not even know whether the evolution was gradual or sudden. As a rule, darwinian selection is more important over long periods of time and Motoo Kimura's picture of molecular evolution by random statistical fluctuation without selection (neutral theory)⁶ is more important over short periods. In reality the evolution must have been a complicated process with incidents of rapid change (punctuated equilibrium — de-

velopment in bursts of evolution)⁷ separated by long periods of slow adaptation.

Finally, one of the persistent concerns of intelligent laymen in Japan since the time of the Meiji Restoration (1868) has been why science in the modern and current sense emerged in a restricted area of the world (Western Europe) and in a restricted period of time (early Middle Ages). It is commonly held that the synthesis of the Judeo-Christian conception of open-ended time and the Benedictine Order's motto "*laborare est orare*" lent progress and activism to the course of the history of Western civilization. We agree with Halstead that one of the secret enclaves of Kyoto University besides coffee houses, the Institute of the Humanities, where Imanishi's basic ideas and concepts took shape, was modelled after a medieval monastery in feudal Europe, though not of genuine Benedictine style.

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Aussie disclaimer

SIR—With reference to the News and Views article by Tony Thulborn entitled "Taxonomic Tangles from Australia" (*Nature* **321**, 13; 1986), I wish to state that *The Australian Journal of Herpetology* and its *Supplementary Series* have no connection whatsoever with any of the *Australian Journals of Scientific Research (AJSR)*.

AJSR is a series of 10 journals published by the Commonwealth Scientific and Industrial Research Organization in collaboration with the Australian Academy of Science. The biological science journals in this series are *The Australian Journal of Botany* and its *Supplementary Series*, *The Australian Journal of Zoology* and its *Supplementary Series*, *The Australian Journal of Agricultural Research*, *The Australian Journal of Biological Sciences*, *The Australian Journal of Marine and Freshwater Research*, *The Australian Journal of Plant Physiology* and *Australian Wildlife Research*.

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Roman Khesin-Lur'e

SIR—Vera Rich, in her comments on the Lenin prizes for science in the Soviet Union (*Nature* 321, 6; 1986), was very sceptical about the merit of this award given to Dr R. Khesin-Lur'e, merely because the award citation dated his publications in biochemical genetics from 1960 when "Lysenkoism was then in full swing". Rich also stated that "it would be interesting to know where Khesin Lur'e published his work at that time, and what part, if any, he played in the overthrow of Lysenkoism". Unfortunately, my old friend R.B. Khesin-Lur'e is unable to answer these questions. The prize was given to him posthumously. Because he was indeed a very prominent scientist, I hope that my brief reply may serve as a short obituary.

Roman Benjaminovich Khesin-Lur'e was born in 1922 and graduated from Moscow University as a geneticist in 1945. His PhD study was carried out under the supervision of the prominent Soviet geneticist A.S. Serebrovsky. In 1948, immediately after Lysenko's "coup", Khesin-Lur'e was dismissed from Moscow University. In 1949–53 he worked as an ordinary technician at the Institute of Biological and Medical Chemistry in Moscow, and in 1954–59 as a lecturer in biochemistry at the Medical Institute in Kaunas. In 1959, he started research work as senior scientist in biochemical genetics at the biological division of the Institute of Atomic Energy in Moscow, where Academician Igor Kurchatov gave protection to many Soviet geneticists who were not able to find work in the academy system or in colleges of higher education. From 1978, Khesin-Lur'e (who is better known in Soviet literature simply as Khesin) worked at the Institute of Molecular Genetics of the Academy of Sciences of the USSR and was, in fact, a founder of this institute.

It is enough to look through *Chemical Abstracts* for 1959–65 to find out that R.B. Khesin was able to publish many of his works during the period of Lysenko's domination. They were published usually in journals, such as *Biochimija*, *Meditsinskaya Khimija*, *Zhurnal Vsesoyznogo Khim. Obshchestva* and in different volumes, such as *Aktual'nye Voprosy Sovremennoi Biokhimii*, starting from Vol. 1 in 1959 and covered many subjects relevant to biosynthesis of proteins and nucleic acids. In 1960, Khesin published his well known (in the Soviet Union) book *Biokhimija Tsytoplasmy* (*Biochemistry of Cytoplasm*), which was published by the publishing house of the Academy of Sciences of the USSR. Khesin-Lur'e did play a very important role in "the overthrow of Lysenko" and did so primarily by his honest and intensive research in an extremely difficult situation. He died from

cancer in 1985, very soon after he was nominated for the Lenin prize.

Lenin prizes in science are not international and the evaluation of scientific merit for them is, of course, a matter for Soviet scientists to decide. The very fact that Vera Rich has apparently never heard the name Khesin-Lur'e before is certainly not sufficient reason to doubt his contribution to science and his integrity as a scientist.

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Environmentalism

SIR—David Pearce rightly criticizes the nuclear industry in his review of Tony Hall's book, *Nuclear Policy: The History of Nuclear Power in Britain*, for having neither the traditions nor the capabilities to handle "environmentalism" when it emerged as a popular and political force. The industry was not alone in failing to appreciate the threat of this movement. It has taken some time for the true motivations of some "environmentalists" to emerge. Many of them seem to be seeking some sort of non-industrial Arcadia irrelevant to the needs of today's world, dominated by the problems of developing countries attempting to achieve some improvement in their living standards in the face of relentless pressures from population growth.

For example, Edward Goldsmith in an editorial in the *Ecologist* asks "How are we to power our expanding industrial society?". He considers the prospects for oil, coal and renewable sources of energy and concludes "The answer is that there is no alternative (to nuclear power)... no nuclear power — no industrial society... the nuclear power station has become the symbol of the industrial way of life... The industrial way of life is squalid, mediocre and unfulfilling. Progress is an illusion."

The consequences of such views are well illuminated by J. Gordon Edwards¹ who points to the enormous amount of damage done by so-called environmentalists using as an example the DDT story, where concern about trace quantities of this material has had untold harmful consequences on malaria eradication programmes.

The world's problems may be soluble through the continuing application of science and technology that has already led to dramatic improvements in infant survival, public health and life expectancy, to a transformation of India from a continent threatened by starvation to a net food exporter, and to the harnessing of nuclear energy to enable the world to survive the depletion of its fossil fuel reserves and avoid the risks of climatic

change from their consumption. They will not be solved by the pursuit of rural Utopias inconsistent with today's population and its aspirations.

The Chernobyl accident should result in a major re-examination of the safety of nuclear plants throughout the world. We must ensure that the lessons of the accident are learned and applied, as happened, at least in the West, after Three Mile Island. But the predictable calls for a run-down of the world's nuclear programmes as a result of Chernobyl is as sensible as a call for the world to do without agricultural chemicals as a result of Bhopal.

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SIR—The leading article "What future for nuclear power?" (*Nature* 321, 367; 1986) contained criticisms of Western environmental movements and impugned the motives of their membership.

Surely imprecation via an examination of motives as a substitute for argument is slightly insulting to the readers of *Nature* or any other journal bent on the promotion of serious discussion.

Apart from the fact that criticism based on an examination of motives is irrefutable in practical terms, it also substitutes mud-slinging for discussion. It is tempting to suggest that the aspiration of the mud-slinger is that some of the mud may stick. But mud-slinging merely obscures issues and never contributes to serious debate.

The Western environmentalist movement has raised serious doubts, based on cogent arguments, about the efficacy and safety of nuclear power. It is these arguments that should be addressed: the reasons why the participants raised them, apart from concern with efficacy and safety, are of little significance.

Focusing on this matter, at the expense of arguing your case, results either from the arrogant assumption that environmentalists are of little consequence because of their populist origins and so their views on nuclear power must also be of little consequence, or a tacit recognition that the environmentalist argument presents problems for those who support the nuclear power programme. If the latter, why not admit it, and if the former, shame on you!

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Looking for gravitational errors

The idea that there may be forces between macroscopic objects other than those caused by gravity is no longer an outrage. But precisely what is meant remains unclear.

THERE is always a special attraction in the discovery in the published literature of a surprising truth that other people have overlooked. This is no doubt one of the extra reasons why close attention was paid to the announcement earlier this year, by Fischbach *et al.* that the Eotvos experiments, first published in the 1920s and widely regarded since then as a proof that the gravitational force between massive objects is independent of their chemical constitution, concealed evidence that there is a force other than gravity between ordinary material objects (see *Nature* **319**, 173; 1986). The proposal is not nearly as heterodox as it may at first have seemed. Most obviously, there have repeatedly been suggestions that the constant in Newton's law of gravitation (called G) is smaller when measured in laboratory-scale experiments than the value required to account for geophysical phenomena.

The article by Fischbach *et al.* originally appeared in *Phys. Rev. Lett.* (**56**, 3; 1986). The essence of its conclusions was that the published errors in the Eotvos measurements of the gravitational attraction between different kinds of substances (carried out with a torsion balance) could be accounted for if there is a force with a characteristic range of about 200 m whose strength depends on the baryon number (only roughly proportional to the mass) of the interacting materials. Now the same journal has published three papers which take the argument a little further, but which have the virtue of suggesting how the matter might be settled.

It is not really surprising that effects such as that described by Fischbach *et al.*, if real, should have been overlooked for half a century. In spite of the exquisite accuracy with which it is possible to describe gravitational systems such as planets in orbit about a central mass such as the Sun, these successes cannot exclude the possibility that short-range forces between material objects may exist. Moreover, in systems such as these, where only relative masses matter to a first approximation, uncertainties may be arbitrarily accommodated in either G or in the planetary and solar masses. This is one reason why it is notoriously a minor scandal that G is, even now, known a little less accurately than one part in 10^5 .

For the sake of clarity, it is worth recalling the form in which the Fischbach result is put. Most simply, G can be set equal to G_0 , the value of the gravitational constant

at infinity, multiplied by a factor differing from one by the addition of a relatively small distance-dependent exponential term of the form $\exp^{(r/l)}$. The distance l then represents the scale over which departures from strict compliance with Newton's inverse-square law may be expected. The supposed constant by which the exponential factor is multiplied is a measure of the strength of the non-newtonian effect. The characteristic length was estimated at 200 m, with a possible error of 50 m in either direction. The constant of proportionality is most distinctive for being negative, meaning that apparently gravitational forces are smaller at smaller distances, or that the extra force is repulsive; its magnitude is such as to affect gravitational attraction by a few tenths of a per cent at zero distance.

It is also, of course, possible that some of the uncertainty in the value of G arises because of attempts to reconcile irreconcilable measurements of gravitational forces on the very large scale and the very small. If, indeed, there is an extra force of interaction perceptible over distances of a few hundred metres only, laboratory measurements of gravitation, going back to Cavendish, will differ systematically from those inferred from astronomical observations. Certainly one of the consequences of the reinterpretation of the Eotvos experiments by Fischbach *et al.* is that future attempts to narrow the errors in measurements of gravitational force will diligently segregate short-range from long-range measurements.

Two of the clutch of three articles now published by *Physical Review Letters* are, like Fischbach *et al.*, of an archival character. Both David A. Neufeld of Harvard University (**56**, 2344; 1986) and S. Nussinov of Tel Aviv University (**56**, 2350; 1986) go back to the records of an experiment by L.B. Kreuzer first published in 1968 (*Phys. Rev.* **169**, 1007; 1968). The objective was to carry out a kind of dynamical equivalent of Cavendish's direct measurement of the gravitational force between two static objects. For this purpose, Kreuzer used as gravitating masses containers with equal volumes of liquid within which were embedded oscillating masses of density equal to that of the surrounding fluid (which is a neat way of ensuring that the centres of gravity of the two objects do not change with the oscillation). The oscillation itself was intended to provide a signal of known frequency

that, with luck and clever design, might be picked out easily from among the inevitable noise. Kreuzer's objective was to distinguish between the "passive" and "active" or dynamic gravitational fields caused by moving masses; his conclusion was that they could differ by, at most, one part in 20,000.

Both Neufeld and Nussinov now point out that the same measurements can be interpreted in terms relevant to the new Fischbach force. For, by good luck, the fluid in the Kreuzer experiment was a mixture of halogen-containing hydrocarbons different in chemical constitution from the solid rods of Teflon suspended in them. The difference is enough to account for a significant difference, for each unit mass, in baryon number, which is a measure of the content of protons and neutrons in a material (whereas the mass of the same material is the same quantity modified by what the nuclear physicists call the packing fraction, or the nuclear binding energy, of the elementary constituents of the materials).

Both Neufeld and Nussinov now argue that the Kreuzer measurements probably provide a more sensitive test of the implications drawn from the Fischbach analysis than an attempt to repeat the Eotvos experiments as such. Indeed, on various interpretations of the Kreuzer measurements, the intermediate-range field of force suggested by the re-analysis is already a little beyond the bounds of likelihood. Neufeld suggests that if the Eotvos measurements are to be repeated, it would be most profitable to do so at the foot of a tall cliff, where the effects of natural gravitational asymmetry would be most apparent.

P. Thieberger from the Brookhaven National Laboratory (**56**, 2347; 1986) is even more lyrical on the subject: repeating the Eotvos measurements at the bottom and the top of a tall cliff could be the most sensitive measurements yet bearing on the existence (or otherwise) of the intermediate force. (The University of Bath, sited on the top of such a cliff, is well placed, among others.) Given this enthusiasm it is certain that there will now be a host of proposals for the re-measurement of gravity by classical devices. The driving force may be the prize of pinning down the intermediate force. It must be hoped that one by-product will be the even more important definition of an accurate value of G .

John Maddox

Immunology

T-cell receptor: gamma gene product surfaces

from Miranda Robertson

THERE are various phenomena in cellular immunology that might demand a second antigen receptor on the surface of T lymphocytes; and as a T-cell specific receptor gene not encoding any part of the known antigen receptor, the gamma gene might have been expected to account for one of them. In fact, however, as has been remarked at intervals in these pages^{1,2}, it has been extraordinarily difficult to find out what the gamma gene is for; and until now, no protein product had been isolated to provide clues. On pages 145 and 179 of this issue^{3,4} two groups report the detection of the gamma gene product in a receptor complex on the surface of human T lymphocytes; and a third paper shortly to appear elsewhere⁵ describes what is almost certainly the same putative receptor but without the evidence that it contains the gamma chain. The data in these reports suggest that the gamma gene is expressed on a small subset of circulating T cells; but there is no obvious way of fitting the gamma chain into any of the immunological phenomena still awaiting a biochemical basis. Before explaining how the receptor came to be detected and why it is difficult to reconcile with any of the roles envisaged for it, I shall briefly recapitulate what is known of the genes encoding the antigen receptor of T cells.

Of three functionally defined subsets of T cells, two — the helper and cytotoxic cells — are now known to express a receptor that is a heterodimer comprising one alpha and one beta chain, each encoded by a gene that is assembled by somatic rearrangement from a large and diverse pool of separate gene segments. This device for generating combinatorial diversity is familiar from immunoglobulin genes and seems to be the hallmark of those surface structures of lymphocytes that are responsible for specific antigen recognition. It is by that criterion alone that the gamma gene has been presumed to participate in the specific recognition of antigen.

The gamma gene was discovered fortuitously in the course of an attempt to isolate the alpha-chain gene (for which it was mistaken), and seems to be essentially similar in structure and organization to the alpha and beta genes, though with significantly more limited variability. There is no firm evidence that its expression is associated with any particular functional subset of T cells; and indeed in most mature T cells it is abortively rearranged — that is, the assembled gene segments

give rise to a transcript that cannot be translated into protein. This and the fact that it seems to be transcribed at relatively high levels early in thymic ontogeny have led several people to focus on the possibility that the gamma gene product has a specific role early in the ontogeny of T lymphocytes when it is believed that T cells 'learn' their unique pattern of dual recognition. The crucial feature of the T-cell receptor is that it is designed, or selected, or both, so as to recognize antigen only when it is associated on the surface of a cell with a molecule encoded by the major histocompatibility complex (MHC). This means T cells must be capable of activation by binding 'self' MHC when it is associated with antigen, but not when it is on its own. If the gamma chain plays a part in the development of this so-called MHC restriction then it is pivotal to the understanding of cellular immunity.

At this point it is only fair to say that the interesting findings described in the three papers I have mentioned earlier give no particular indication of any such role. The findings themselves are as follows.

The assumption from which all three groups started was that any antigen receptor on a T cell would probably be co-expressed with T3. T3, a multi-chain complex expressed on all T cells, is believed to be responsible for transducing the activation signal when the receptor heterodimer binds antigen. It is closely but non-covalently associated with the alpha-beta heterodimer and any mutation that prevents the appearance of the receptor heterodimer also prevents the appearance of T3 (ref. 6). Nonetheless, there are lymphocytes that express T3 in the absence of the alpha and beta chains: they seem to account for about 3–8 per cent of peripheral blood lymphocytes (PBL)^{3,5}. The presumption is that in these cells T3 may be co-expressed with some other receptor, for which indeed some evidence has already been reported⁷.

The biochemical definition of the receptor complex has however become possible only with the availability of large numbers of these relatively rare cells. As they report in this issue, Brenner *et al.*³ succeeded in growing two T3⁺ alpha-beta⁻ cell lines from the PBL of immunodeficient patients (in normal individuals they are rapidly overgrown by alpha-beta⁺ cells); also in this issue, Bank *et al.*⁴ report the cloning of a cell with this phenotype after having selected for T4⁺T8⁻ cells

(believed to be immature thymocytes); and Weiss *et al.*⁵ found a T3⁺ alpha-beta⁻ leukaemia. Much the most detailed biochemical analysis is that of Brenner *et al.*, who report that their cell lines (which are 50 per cent T4⁺T8⁻ and 50 per cent T4⁺T8⁺) express T3 in association with two chains: one of relative molecular mass 55,000 (55K) that binds antibodies raised against synthetic peptides representing gamma gene sequences; and a 40K peptide which they designate the delta chain. Bank *et al.*⁴ have a heterodimer in which a 60K protein is associated with a 44K chain that is recognized by the anti-gamma antibodies; and Weiss *et al.*⁵ find only a single 55–60K chain which they have not yet tested for reactivity with anti-gamma antibodies.

Clearly there are some discrepancies in the data from the three sources; but they are probably trivial. The difference in the size of the gamma chain identified by Brenner *et al.* and that of Bank *et al.* may for example be due to differences in glycosylation. On the other hand, the failure of Weiss *et al.* to find a second chain for their putative receptor is not surprising: it is not always easy to detect both chains of the established alpha-beta heterodimer; and besides, Brenner *et al.* and Banks *et al.* find that the gamma chain is not disulphide-linked to the second putative receptor chain. This distinguishes the new receptor complex from any other antigen receptor and may encourage speculation about the formation of alternative complexes between gamma and, say, beta chains; which brings us to the question of what the receptor is for. Only Bank *et al.* have functional data: they find that anti-T3 antibodies can induce both cytotoxicity and interleukin-2 secretion in their cloned cells, which thus seem to have the expected attributes of a normal T cell.

There are two cell types still in search of a receptor: natural killer (NK) cells, which may be related to T cells; and suppressor T cells. Do these data fit either of them? NK cells, like the cloned cells of Bank *et al.*, have a T8⁺T4⁻ phenotype and they are of course cytotoxic; but there is good evidence⁸ that they do not transcribe gamma genes.

Suppressor cells belong to an elusive regulatory subset of T cells that do not express the alpha-beta heterodimer that is common to the better-established helper and cytotoxic subsets. Might a gamma-delta complex be the suppressor receptor? Because of the rather ill-defined nature of this subset it is hard to rule this possibility out; but suppressor cells might be expected to constitute more than 3–8 per cent of PBL, and there is no obvious reason why, if cytotoxic and helper cells can use the same pool of receptor genes, suppressor cells should need a different one.

Finally, what of the postulated role of the gamma chain in T-cell ontogeny? It

cannot be excluded. But it is very difficult to imagine how an immature thymocyte expressing a given gamma-delta heterodimer could be selected for the properties of a subsequent alpha-beta heterodimer; so gamma would have to be co-expressed for at least some of the time with alpha or beta or both (and indeed a scheme of this sort is envisaged in one of the theories on the early role of gamma⁹); however there is no evidence that this occurs and Brenner *et al.* and Bank *et al.* know there are no functional alpha or beta transcripts in their cells while Weiss *et al.* find beta transcripts but there is no evidence that they are functional. It remains possible nonetheless that the alpha and beta genes are functionally rearranged later and a two- (or one-and-a-half¹⁰) receptor system might operate at some stage in ontogeny.

Co-expression of gamma and the alpha-beta heterodimer as the basis for dual recognition of antigen and MHC in mature T cells¹¹ can probably be excluded

because dual specificity for MHC and antigen can be conferred by transfection with the alpha and beta genes alone¹¹.

One would have thought there was already quite enough cellular immunology to satisfy any demand for a phenomenon to fit a molecule; but it begins to look as though to understand the gamma gene it may be necessary to invent still more of it. □

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Particle physics

Particles and the Universe

from Neil Turok

COSMOLOGY is now the main arena in which fundamental theories of particle physics can be tested — an exciting stream of data on the large-scale structure of the Universe is just beginning to appear. It is also interesting that particle physics experiments designed to detect the 'dark' matter in the Universe directly should become operational in the next few years. Developments in these areas were discussed at a recent meeting*.

The most recent observations support earlier indications that the Universe has a lot of structure on quite large scales. This is very important for theories of particle physics. In most theories the matter has not moved about much on these scales since the Big Bang and so the observations provide a fairly direct 'window' on what the very early Universe was like.

Five years ago, the Bootes void, a region about 60 Megaparsecs (Mpc) across apparently devoid of bright galaxies was discovered. On the average, such a region would contain a thousand galaxies. More recently it was found that clusters of galaxies are themselves significantly clustered on a similar scale. Now two further observations support the existence of large-scale structure.

The first, discussed by Joseph Silk in a recent *News and Views* article (*Nature* **320**, 12; 1986), is the result of measuring redshifts of galaxies in a thin slice of the Universe out to 150 Mpc. The measurement reveals a 'foamy' distribution of

galaxies, most of them lying on sheets surrounding large, almost empty holes of radii up to 50 Mpc. A problem with these data is that no correction has been made for peculiar velocities — the redshifts are simply interpreted as being the result of Hubble flow. Peculiar velocities produced by local gravitational infall can produce such sheets as artefacts. But even if this is the explanation, the data seem to indicate large coherent velocities in the distribution of galaxies over large scales.

The second observation confirms this point of view. Richard Bond and Sidney van den Bergh described in a recent *News and Views* article (*Nature* **320**, 489; 1986) a large region of radius about 50 Mpc around our Galaxy that appears to be moving coherently with a velocity of about 1,000 km s⁻¹ relative to the microwave background. This is a significant fraction (about a fifth) of the Hubble flow on that scale.

These observations are bad news for the most popular current theory of the origin of fluctuations in the Universe. This is based on the notion of 'quantum fluctuations' produced by a field in the process of causing an inflationary stage in the very early Universe. One of the early successes of the inflation model was that it predicted a reasonable spectrum, the Zeldovich spectrum, for the fluctuations. However, inflationary models require an artificial adjustment of parameters to very small values to give the perturbations a reasonable amplitude. There was consensus at the meeting that although inflation re-

mains a very good idea, no aesthetic model in which it is realized has yet been devised.

Quantum fluctuations have several attractive aspects for cosmologists. They are linear, adiabatic and have a gaussian probability distribution. So it is comparatively easy to calculate their effects. Inflation clearly predicts that the cosmology density parameter Ω is very close to unity. In conjunction with arguments from nucleosynthesis, this requires the Universe to be dominated by non-baryonic matter.

The first models to be looked at in detail were universes dominated by hot dark matter such as massive neutrinos, which free stream out of dense regions and erase perturbations on the scale of galaxies. As a result galaxies form much too late. Hot dark matter models appear to predict fluctuations in the cosmic microwave background marginally larger than the observational upper bounds (G. Efstathiou, Cambridge). But some criticism was made of the statistics used in obtaining the bounds (F. Melchiorri, Rome); there is agreement that the bounds usually quoted are too low by a factor of at least two.

The next models were universes dominated by cold dark matter, for example, axions, photinos or quark 'nuggets'. These models have the opposite problem (J. Primack, Santa Cruz). Although galaxies formed early enough, there is very little structure on large scales — in particular the correlations between clusters of galaxies are hard to understand. One way out is to invoke biasing — the idea that visible matter (stars) formed only at the peaks of the underlying matter distribution. Galaxies formed above some threshold, clusters formed at a higher threshold and so on. With a gaussian distribution, this increases the correlations of clusters, although not on scales as large as 50 Mpc. Voids are another possibility. Here, the large-scale structure appears as a statistical artefact of our seeing only a small fraction of the matter in the Universe. The underlying distribution of matter is very smooth, and coherent velocities on the scales which have now been observed were less than 160 km s⁻¹. With a large degree of confidence, these models now seem to be ruled out.

The main alternative theory of the origin of structure involves cosmic strings, relativistic defect lines which are predicted to form in the early Universe by many Grand Unified Theories and superstring theories. Loops of cosmic string chopped off a network seem to have just the right properties to seed the formation of galaxies and clusters of galaxies (see Hogan, C. *Nature News and Views* **320**, 572; 1986). Interestingly, cosmic strings are able to produce the required small- and large-scale structure in either hot or cold dark matter universes. In the former, small

* Second ESO/CERN symposium: Cosmology, Astronomy and Fundamental Physics, Munich 17-21 March 1986.

loops of string survive the free streaming and go on to seed galaxies, whereas in the latter, larger loops provide the large-scale structure. Cosmic string theories produce a correlation of clusters of galaxies remarkably similar to what is observed. Detailed calculations of the large-scale coherent velocities produced by cosmic strings have yet to be made. With hot matter these will almost certainly be large enough, and even with cold matter the fluctuations on large scales caused by large loops are much larger than with quantum fluctuations.

There remains the crucial question of the dark matter. Evidence from galactic rotation curves and from the dynamics of galaxy clusters suggests that dark matter does exist, but this is quite consistent with the simplest explanation: an $\Omega = 0.1$ Universe dominated by large planets (Jupiters) (M. Rees, Cambridge). But recent results from the infrared survey IRAS give a measurement of Ω on very large scales of around unity (M. Rowan-Robinson, Queen Mary College, London). If confirmed these results will reinforce ideas of the fundamental simplicity of our Universe, and the question of what the non-baryonic matter is will become more urgent.

Particle physics has already come up with a plethora of candidate particles for the non-baryonic matter, none very well motivated. The massive neutrino received a lot of interest after Lyubimov *et al.* (*Phys. Lett.* **94B**, 266; 1980) reported a mass for the electron neutrino in the cosmologically interesting range, around 30 eV, some time ago. However, a recent

experiment using more sophisticated apparatus disagrees with this value, setting an upper bound of 18 eV on the electron neutrino mass (K. Winter, CERN). The possibility remains that the Universe is dominated by the (very likely heavier) muon or tau neutrinos.

Some very interesting new ideas for detecting cold dark matter particles have been proposed recently (P. Smith, Rutherford Appleton Laboratory). The axion is probably the best candidate, being very light (about 10^{-5} eV) and very weakly coupled to other matter. Axion decay into two photons can be stimulated by a tuned oscillating field in a resonant cavity, and several experiments to detect axions by this method are under way.

Small pure crystals or lumps of superconducting metal can be used to look for more massive (about 10–1,000 GeV) weakly interacting particles like photinos or sneutrinos. Such a particle colliding with 1 mm³ of silicon at 50 mK would cause a recoil in the nucleus it collided with sufficient to heat the crystal by 5 mK. With existing technology, this can be detected with an accuracy of 1 per cent. A detector containing 1 kg of such crystals would be expected to see from 1 to 1,000 events per day if these particles dominated the Universe. The experiments will probably take 2–3 years to set up and will open up a new area of particle physics; at the very least they will provide invaluable information on what the dark matter is not. □

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being applied to burnt flint, and to wind-blown sediments: these applications offer new opportunities for dating on Palaeolithic sites, and are particularly useful beyond the present precision of radiocarbon (~40,000 years before present). One of the first examples was the dating of two burnt flints from the site of Terra Amata, Nice, to 230,000 years before present⁵.

Aerial photography on various scales is being intensively used by archaeologists. Satellite photographs are generally on too large a scale to be useful, but LANDSAT images have been used to trace broad aggradational features (ancient levee systems) in Mesopotamia⁶. Below-ground features showing up on oblique aerial photographs can now be mapped more precisely by using relatively simple computer programs, for instance, multi-point projective transformation⁷. Geophysical instruments have been refined and developed, and include soil-sounding radar, which has been tested at Sutton Hoo, and side-scan sonar, used in underwater archaeology⁸. Detailed air photographs or geophysical plots have increasingly been used as the basis for archaeological interpretation, since they are less costly than excavation. Although this is a valid exercise, especially for examining large landscapes, such interpretations need to be checked by excavation.

Where excavation does occur, automatic recording of information on microcomputers is becoming more widespread, and topographical survey before and during excavations has been revolutionized by the electronic distance meter. In 1984 this instrument was used to survey a Lincolnshire long barrow and adjacent excavation trench in only four hours.

Studies of past environments, in terms of soils, sediments, pollen grains and molluscs, are a standard feature of site investigations of all time periods. Increasingly sophisticated and precise techniques have been developed (for example, soil thin-sectioning and diatom analysis).

All the structures which archaeologists excavate, from scarcely visible shadows of postholes and pits to walls of brick or stone, can be characterized or fingerprinted scientifically, although frequently the lack of funds prevents this from taking place. Some techniques, such as thin-sectioning or physical chemical analysis, are well established, but need to be expanded to all relevant raw materials in buildings and other structures.

New methods are also needed to study the composition of common artefact types, such as flint tools or some types of pottery, where extensive work using atomic absorption spectroscopy or neutron-activation analysis has only been partially successful in separating out source materials. Early metal artefacts are also difficult to characterize satisfactorily. However, their composition is becoming

Archaeology

Towards a scientific approach

from Patricia Phillips

ARCHAEOLOGY, the study of our past, developed in a humanistic framework, although basic methods such as observation of stratigraphy were borrowed from contemporary sciences. Recently, more sophisticated techniques have been developed and refined to provide a basis for many types of archaeological investigation. But in the United Kingdom more money is needed to keep pace with current excavations and fieldwork and to apply the new techniques to the many artefacts and ecofacts generated by government-funded programmes over the past two decades. This is the message of a new report — archaeology must be science-based in fact, not just in name.

Many archaeological finds, from whole sites to individual buildings, early industrial structures and assemblages of artefacts and ecofacts, fail to be exploited to their full scientific potential. Much more

use should be made, both of established scientific techniques and of newly developed methods, to maximize the information from each archaeological find.

Readers of *Nature* will be familiar with some of the more recent applications of science to archaeology, such as refined radiocarbon measurement techniques permitting very small samples to be accurately dated. At the accelerator laboratory in Oxford, individual charred wheat grains from Danebury hill fort, England and from Mycenaean Greece have been successfully dated¹. The European tree-ring chronology, based mainly on German and Irish oaks, can be traced back more than 7,000 years², and small sequences of tree rings have been used to 'recalibrate radiocarbon dates from the Neolithic period onwards'. Another dating technique, thermoluminescence, originally used on ceramics and ancient hearths, is

better understood, both as larger series are investigated and with the application of lead isotope analysis⁹. This relatively new method can identify the sources of ancient coins and metal artefacts. More recently, inductively coupled plasma spectroscopy has been used to characterize stone, pottery, metal and glass.

During the 1970s and 1980s the scanning electron microscope and its auxiliary tool, the electron microprobe, were increasingly used to investigate the structure and composition of archaeological artefacts and ecofacts, from crucibles and coin moulds to fragments of wood scabbard and sheepskin scabbard lining¹⁰. The scanning electron microscope is also useful as an addition to standard microscopy in microwear analyses. These studies of the polishes, striations and residues which indicate how a tool was used, and on what material, have expanded widely in western Europe since the mid 1970s. Although the high magnification of the analyses (at least 280 ×) restrict the numbers of artefacts that can be studied, the method is objective, and has allowed significant progress in the study of stone and bone tools. For instance, the method demonstrated that flint flakes found beside a Neolithic trackway in the Somerset Levels, United Kingdom, were used to cut reeds and whittle wood¹¹.

New methods are being developed to age skeletons¹² and to determine past diets. Blood grouping on ancient bone, to identify relationships between ancient mummified or buried bodies, a widely exploited technique, particularly in Hungary, has been applied to few individuals from British tombs. However, the publicity surrounding 'Lindow Man', whose 2,500-year-old body was found in a Cheshire peat bog, enabled a wide range of tests to be applied (from standard X rays and scanning electron micrographs to Xero radiographs, nuclear magnetic resonance, computer tomographic scans and surgery using a fibre optic endoscope)¹³. □

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Parasitology

Clandestine sex in trypanosomes

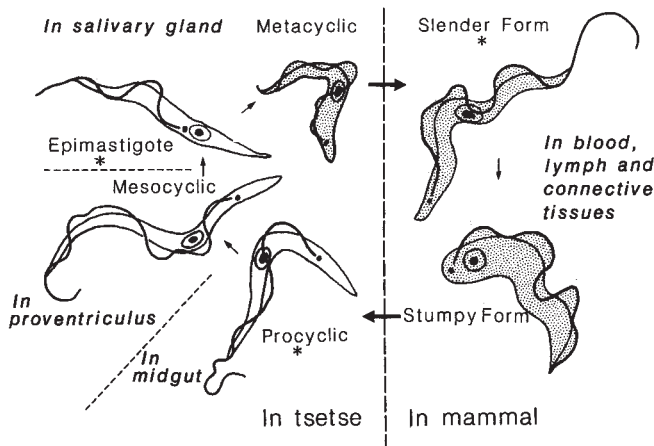
from Keith Vickerman

ALTHOUGH few biologists would question that eukaryotic organisms once needed sex to reach levels of cell complexity denied to prokaryotes, there is room for doubt over whether many unicellular eukaryotes have had cause to retain it. Indeed there is a complacent conviction among those who study them that a haploid genome and short doubling time enable the asexually reproducing protist to look natural selection in the face — and survive without the gene-shuffling benefits of meiosis and syngamy. So the demonstration of hybrid formation in sleeping-sickness trypanosomes after transmission by the tsetse fly vector, described on page

A further source of genetic variation in trypanosomes may lie in the kinetoplast (mitochondrial DNA) close to the base of the flagellum (see figure). This unique structure contains about 100 20-kilobase (kb) maxicircles (corresponding to the mitochondrial genome in other eukaryotes) interlocked with several thousand 1-kb minicircles whose sequences alter during vegetative growth, providing another possible system of genome reassortment. The function of the minicircles (other than maintaining the network) is a complete mystery. Maxicircle deletions or disruption of the network are associated with loss of ability of the single mitochondrion,

which is repressed in the mammalian host, to become activated in the vector³.

Trypanosomes ingested by the blood-feeding tsetse fly embark on a long and complicated cycle of development and multiplication, first in the vector's gut and later in its salivary glands (see figure)⁴. Hitherto the trypanosome cycle has revealed no obvious gamete or zygote stages. Yet Jenni and colleagues find that



Developmental cycle of *Trypanosoma brucei*. Shaded areas, stages expressing variable antigen genes; asterisks, multiplicative stages.

173 of this issue, exposes a surprising and hitherto clandestine activity on the part of these important parasites, and one which calls for reassessment of several aspects of their biology.

One reason for doubting the existence of sexuality in trypanosomes was that these organisms have their own ways of generating genotypic diversity, regardless of sex, and these have received much attention in recent years. The tsetse fly-transmitted parasites have met the principal challenge to their survival in the blood of an antibody-producing host by evolving an elaborate genetic mechanism for repeatedly changing the antigenic nature of their protective glycoprotein coat⁵. Only one of 1,000–2,000 variable antigen genes is expressed at a time and expression can occur only at a chromosomal telomere. The switching process usually involves DNA rearrangements in order to get the new gene into the telomere expression site. These rearrangements provide a system of recombination and reassortment of variable antigen genes during asexual multiplication.

The mixture of two parental trypanosome stocks followed by tsetse transmission can result in stocks of non-parental phenotype which are heterozygous for parental-homozygous isoenzyme and variable antigen gene restriction fragment markers. These findings come some years after the observation by Tait⁶ that the frequency of enzyme electrophoretic variants in a natural population of *Trypanosoma brucei* conforms to the Hardy-Weinberg equilibrium, suggesting that mating and meiosis occur, and that allele segregation takes place in an orthodox mendelian fashion. Bloodstream *T. brucei* appear to be diploid⁷, so if this gene-exchange system is a conventional one meiosis and 'gamete' formation must take place in the vector.

But where in the fly does this clandestine sex occur? The most likely place would be in the salivary glands where the attached epimastigote forms are crowded together under conditions of extreme intimacy; the epimastigote might perhaps furnish gametes, the non-dividing mammal-infective metacyclics derived from

them might be zygotes.

The question of trypanosome ploidy will become more confusing with a further publication from groups in Basel and Brussels reporting that Feulgen spectrophotometry gives a metacyclic nuclear DNA content which is half that of the bloodstream form⁷. The obvious interpretation is that haploid metacyclics give rise to diploid blood infections after mating in the mammalian host. This seems unlikely. Finding a mate would be more difficult in the mammal than in the fly. Furthermore, metacyclic-derived clones from heterozygous stocks fail to give rise to homozygous recombinants in blood infections, as they should if metacyclics are haploid, but remain heterozygous⁸. One possible interpretation of the data presented in this issue, however, is that the hybrids are tetraploid. In order to settle this point their DNA content should now be compared with that of parental stocks.

Clearly, much more work is needed to characterize the new genetic system and the first step should be examination of the progeny from reciprocal back crosses and the generation of an F₂ by mixing different hybrid clones to establish that classical meiosis and recombination occur. So far meiosis appears to be confined to eukaryotes which have conventional mitosis, while trypanosome nuclear division with its non-condensing chromosomes and peculiar spindle apparatus is highly unconventional.

The technique of pulsed-field gradient electrophoresis used for separating small chromosomes has revealed an extraordinarily large number of chromosomes in *T. brucei*: in addition to several 2,000-kb chromosomes there are about six of intermediate size (200–700 kb) and about 100 minichromosomes (50–100 kb)⁹. This multitude of chromosomes appears to provide an abundance of telomeres for expression of variable antigen genes. How the countless daughter chromosomes segregate at mitosis is difficult to imagine as electron microscopy shows that the intranuclear mitotic spindle contains only a few microtubules and less than 10 of the structures identified as kinetochores¹⁰. Not surprisingly, the intermediate and minichromosomes show considerable variation in size and number between trypanosome stocks and even size variation during successive generations of a

clone. Using existing hybrid progeny it should be possible to examine the inheritance of chromosome variation and of kinetoplast DNA; in the maxicircles of the latter, restriction enzyme polymorphisms have already been identified, so markers are available¹¹.

The demonstration of sexuality in *Trypanosoma brucei* is of importance in the interpretation of epidemiological data. In epidemics of acute sleeping sickness several zymodemes (parasite populations distinguished by their isoenzyme profile) are found in the human population and it now seems possible that such epidemics might arise as a result of genetic exchange between existing zymodemes¹². It will be interesting to see if *T. brucei* throughout Africa is divided into

reproductively isolated populations. Experimentalists will want to know whether there are restrictions to hybridization, for example, the occurrence of mating types common in other eukaryotes; whether trypanosome sexuality is associated only with late-stage fly infections (most of our knowledge of development in the vector comes from early-stage infections); and whether differences in nuclear DNA content are simply a matter of ploidy or whether gene amplification and chromosome diminution also play a part. Undoubtedly, further surprises are in store for us! □

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Meteorology

European collaboration on dynamic atmospheric fronts

from K.A. Browning, B.J. Hoskins, P.R. Jonas and A.J. Thorpe

METEOROLOGY is a science that depends on the collaborative links between those involved in developing basic theories and in data collection, analysis and forecasting. The European Mesoscale Frontal Dynamics Project, an intensive programme for observing and modelling active cold fronts passing over north-west Europe, should improve both our understanding of frontal structure and the predictions of the associated mesoscale rainfall distributions. The observational phase of the project is planned for the autumn of 1987, and until then theoretical and modelling work will attempt to define precisely the type and scope of the data to be collected. The project will include university groups, national meteorological offices and research organizations in the United Kingdom, France and the Federal Republic of Germany.

An atmospheric front marks a transition zone between air of markedly different properties, such as temperature or moisture content (see Fig. 1). It is remarkable that air flow should generate such sharp zones, with horizontal scales in the atmosphere of only a few tens of kilometres, rather than maintaining a weaker, broader thermal contrast between equator and pole. In the terms of this definition, frontogenesis — the formation of fronts — is a fundamental fluid dynamical problem. Because rain and snowfall are concentrated at fronts, the problem is of considerable meteorological importance. It is now believed that one of the major challenges in weather forecasting is to predict the variations of precipitation on space and timescales of the order of a hundred kilometres and a few hours that

are typically associated with fronts in mid-latitudes. Such mesoscale variations are intermediate between the 1,000-km scale of depressions and the 10-km and smaller scales of individual convective clouds. Although predictions of the large-scale patterns of depressions and anticyclones are

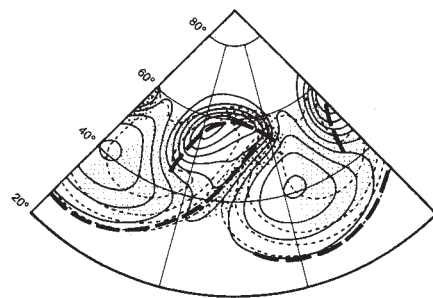


Fig. 1 Model simulation of an atmospheric frontal system¹³. Solid lines, surface pressure contours every 8 mb; dashed lines, low-level temperature contours every 8 K. Areas of strong ascent and descent, respectively, are shown heavily and lightly stippled. Heavy dashed lines, maximum in the low-level vorticity, corresponding to the position of fronts.

now rather accurate even for four days ahead, it is difficult to predict accurately the smaller storms and bands of rain embedded within depressions.

The influential 50-year-old Norwegian model^{1,2} of fronts is now thought to be misleading in several respects: for example, it imagines a depression as forming at a pre-existing frontal discontinuity whereas the modern view is that fronts form after a major depression or cyclone starts to grow. In other words, frontogenesis is now generally thought to follow

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cyclogenesis rather than vice versa. But the model still holds in some situations — for example, when mature fronts approach the north-east Atlantic, a wave cyclone of scale 200 km can develop on a cold front, producing an intensification of rainfall over north-west Europe. The reasons for the generation of these waves will be investigated by the new project.

There have been several significant advances since the Norwegian model was formulated. First, the westerly flow in mid-latitudes was shown to be unstable, leading to the periodic growth of the familiar depressions or cyclones. This 'quasi-geostrophic' theory^{3,4} predicts rather accurately the horizontal scale and rate of growth of these systems. Although it suggests that in certain regions of the cyclone the conditions are favourable for the formation of fronts, it predicts that it would take an infinite time to produce the observed near-discontinuity. The formation of a front in a short time interval (of the order of one day) was shown to be caused by a non-linear feedback mechanism at the front⁵, neglected in the quasi-geostrophic model. This feedback can be described approximately as follows. As air rises just ahead of the front, its rotation about a vertical axis increases essentially to conserve the fluid dynamical analogue of angular momentum. This increase in rotation forces more low-level convergence and hence more ascent. An assumption of this 'semi-geostrophic' model is that the flow remains in a balanced state; that is, the role of high-frequency motions remains merely one of adjustment of the mass and the wind field to each other.

Where, then, are the essential missing links in present-day understanding of the processes responsible for the formation and structure of fronts? The major factor omitted in the semi-geostrophic model is the role of moisture and the consequent clouds and precipitation. Ideas in the past 5 years concerning the basic structure and dynamics of fronts have led to some insight into the production and organization of frontal rainfall⁶. The main questions still to be answered by the new project concern interactions between the processes

responsible for rainfall and those ultimately determining the development of the front.

Other aspects that will be investigated include the assumption that frontal regions are one of the most active parts of the atmosphere with respect to the exchange of air (and pollutants) between the stratosphere and the troposphere. The tropopause, the base of the stable stratosphere, is typically at a height of about 10 km above the surface, but it becomes highly distorted locally in frontal zones. Such are the vertical circulations at fronts that the tropopause has been observed to descend to within 3 km of the Earth's surface as a narrow 'intrusion'. Within the intrusion relatively dry and stable stratospheric air is brought far down from its normal location, to be mixed into the tropospheric air. The relative position of the intrusion with the surface front is thought to help to determine the degree of development or suppression of the convection that produces localized areas of heavy frontal rainfall. It is possible to track the position of the intrusion by monitoring tracers such as ozone or water vapour, using satellite or aircraft-borne detectors.

An essential hypothesis of semi-geostrophic theory, that of balanced flow, has recently been questioned — see the *News and Views* article by K. Emanuel⁷, who reported suggestions that frontogenesis is halted if the flow becomes unbalanced, that is, if the operation of the non-linear feedback mechanism leading to the sharp front is prevented by the smaller-scale motion at the front. But recent high-resolution observations and modelling work do not seem to support this idea^{8,9}.

A special field programme is needed because the theoretical models cannot be tested using only the data collected by routine weather-observing networks. These observations of temperature, humidity and winds in the upper air have a horizontal resolution of about 300 km, whereas fronts may be characterized by a scale of only 50–100 km. The weather radar networks currently being developed over much of western Europe can provide rainfall data and an indication of the presence of dynamical features over a wide range of scales from hundreds of kilometres down to less than 10 km, but these observations are not sufficient to define the dynamical and thermodynamical structure. Satellite cloud imagery also provides some information on cloud structure down to small scales but it gives limited quantitative information on atmospheric structure (see Fig. 2).

The planned experiment will focus on the western parts of the English Channel, southern England and northwestern France, where fronts approaching from the west (the dominant direction of movement) will be relatively unaffected by lift-



Fig. 2 Satellite photograph (NOAA-9 visible imagery) of the cloud patterns associated with an atmospheric frontal system broadly resembling that modelled in Fig. 1 (courtesy of The University of Dundee).

ing over hills. It is intended that the conventional upper-air observations over the area of the experiment will be enhanced for short periods centred on the passage of fronts.

Extra observing systems will be used to obtain data over the range of scales required. These systems include specially instrumented aircraft such as a Dornier 128 (ref. 10) operated by German scientists to measure winds and turbulence, and a Hercules aircraft, operated by the UK Meteorological Office¹¹, with the capability of ejecting dropsondes that can make frequent measurement profiles of temperature, humidity and wind. Various kinds of ground-based Doppler radar, operated by French scientists¹², will provide detailed three-dimensional wind fields within areas of rainfall as well as continuous wind profiles within the clear air. Powerful computing facilities will support the running of mesoscale forecast models both in the United Kingdom and France. These models are still in their infancy and it is expected that the data sets obtained in the new project will enable their basic hypotheses and predictions to be tested and improved. □

Erratum

In the article by Frank Westheimer (*Nature* **319**, 534; 1986) references 8 and 9 should have read: 8. Milstein, S. & Cohen, L.A. *J. Am. chem. Soc.* **94**, 9158 (1972); 9. Modrich, P. & Zabel, D. *J. biol. Chem.* **251**, 5866 (1976). Professor Westheimer's permanent address is: Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138, USA.

Corrigendum

In the article 'The class number problem' by Ian Stewart (29 May p. 474) the example of Fermat's theorem that "every prime of the form $6n+1$ can be written as x^2+3y^2 for integers x and y " should have read: 31 is such a prime, and $31 = 2^2+3.3^2$.

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Nuclear magnetic resonance

First sight of a single cell

from John Mallard

A PAPER on page 190 of this issue¹ describes the first nuclear magnetic resonance (NMR) images of a single cell. This offers the exciting possibility of fine-resolution NMR imaging of cells and collections of cells. The magnetic-field strengths which are used in clinical imaging range from 0.02 to 1 tesla, with about 0.1–0.5 tesla being the norm, but by going to 9.4 tesla and limiting the volume to be studied to a couple of hundred micrometres in thickness, the authors achieve spatial resolutions in the image of the order of 10 to 30 micrometres.

There was much excitement in 1980 when the first clinically useful images of the thorax and abdomen of a patient with multiple cancer deposits were obtained using the technique of NMR imaging^{2,3}. This was the realization of a goal first mooted in the early 1970s (refs 4,5) and pursued in several laboratories since then. By 1984 more than 200 machines were being used in hospitals⁶, with 15 companies manufacturing them.

Nevertheless, I believe that the full potential of this powerful new imaging technique has still to be realized. What is being imaged is the hydrogen protons of water (and fat) in the body, and the parameters which contribute to the formation of contrast in the image are the proton density (or water concentration); the two magnetic resonance relaxation times T_1 and T_2 ; and the proton movement. The time T_1 is dependent on the interplay of the water molecules and their macromolecular environment, while T_2 is related to the interplay of the water molecules among themselves, and by carefully choosing the sequence, timing and duration of magnetic-field gradients and radio-frequency pulses one can tailor the image to emphasize one or other of these parameters to emphasize one or other aspect of water use in that tissue being imaged.

Because some of the images show vivid anatomical detail of soft tissue, such as the brain, they are immediately compared by radiologists with the images obtained by the X-ray methods, particularly X-ray tomography or X-ray scanning. Naturally, much effort is now being expended in finding which regions of the body and which disease or which abnormality this new imaging technique is better or worse at than these other existing procedures.

Although this exploratory stage is necessary, it is important not to lose sight of the fact that the signals forming the image are from a quite different source than those from X-rays. The X-ray images are formed by differential absorption of

X-rays by the electron clouds of the larger, heavier atoms in the body, whereas these new signals come largely from the nuclei of the hydrogen atoms of the water in the body. The movement of the protons in and out of the imaging volume also influence the signals, and there is now much effort to harness this to show blood flow in major vessels and even to measure the amount of flow, and deviations from it, in these vessels.

No doubt we will now see a rapid exploration of the new NMR instrument described in this issue and a comparison of what it can do with the other micro-

scopic techniques. At first sight this will include the study of material which is alive, and perhaps the really new distinctive feature may be the imaging of the dynamic processes of tissue fluid flow and diffusion. Might not the knowledge gained from the NMR microscope lead us back into attempting to obtain safely a much finer spatial resolution in clinical images than can be achieved now? Might it also help us to understand the really new contribution of NMR to medicine? □

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Earth sciences

Anoxic oceans and short-term carbon isotope trends

from Wayne D. Goodfellow

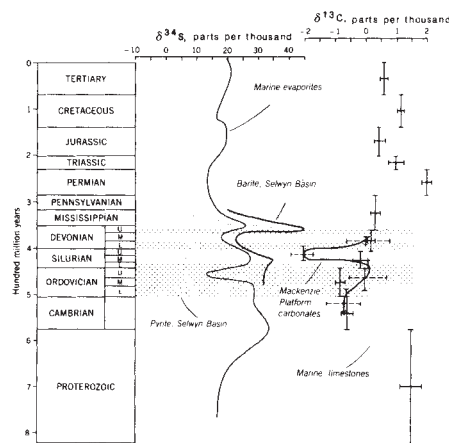
THE relationship between catastrophic geological events, mass mortality of species and sudden shifts in carbon isotope levels was demonstrated for the first time at the Cretaceous–Tertiary mass-extinction boundary, where negative $\delta^{13}\text{C}$ values (see figure legend) were found to be associated with an iridium anomaly. Similar relationships between platinoid anomalies and short-term carbon fluctuations have been shown at the Permian–Triassic², Frasnian–Famennian³, Precambrian–Cambrian⁴ and, most recently, Proterozoic–Palaeozoic⁵ boundaries. At other stratigraphic boundaries, a shift in carbon isotope values has been observed with no associated metal anomalies⁶. Many of these changes have been attributed to reductions of the biomass, although other factors may be important.

Changes in carbon isotope values in marine carbonates are influenced by many factors, such as the level of mixing of the oceans, fractional preservation of organic matter and biological productivity. Changes in carbon fluxes with time are also important although they are unlikely to explain the relatively short-term and sudden decreases recorded at some System boundaries.

Because living organisms preferentially accumulate carbon-12, an increase of biomass is reflected by a corresponding proportional decrease in the carbon-12 in dissolved carbonate. The converse is also true — a decrease in biological productivity is recorded by an increase in the carbon-12/carbon-13 ratio. Although

there is general agreement that perturbations in productivity can generate changes in carbon isotope ratios in carbonates, the cause(s) of these shifts at a particular horizon is often unclear.

The recent data of Magaritz *et al.*⁵ were obtained from a section from the Siberian Platform spanning the Proterozoic–



Short-term $\delta^{34}\text{S}$ trends in pyrite and barite from the Selwyn Basin⁹ and global evaporites¹⁰ and corresponding curves for $\delta^{13}\text{C}$ changes in carbonates from the Mackenzie Platform and global carbonate¹¹. Small dots, ventilated water column; large dots, water column stratified with anoxic waters.

$$\delta^{13}\text{C} = \left[\frac{^{13}\text{C}/^{12}\text{C sample}}{^{13}\text{C}/^{12}\text{C standard}} - 1 \right] \times 1,000 \text{ parts per thousand}$$

$$\delta^{34}\text{S} = \left[\frac{^{34}\text{S}/^{32}\text{S sample}}{^{34}\text{S}/^{32}\text{S standard}} - 1 \right] \times 1,000 \text{ parts per thousand}$$

Palaeozoic boundary. The $\delta^{13}\text{C}$ values increase 15 m below the Vendian–Tommotian boundary and decrease at the boundary. The authors interpret these changes as a reflection of an initial bloom in biomass followed by a decrease in productivity on the basis of two lines of evidence. First, the association of highly negative $\delta^{13}\text{C}$ values with the Vendian–Tommotian boundary indicates, by analogy with negative isotope shifts observed at other system boundaries, a decrease in productivity; and second, a major phosphogenic episode near the end of the Precambrian is evidence for increased productivity, which perhaps explains the trend to more positive $\delta^{13}\text{C}$ values during most of the Vendian.

One of the problems with this approach is that the Precambrian–Cambrian boundary has not yet been defined and the Vendian–Tommotian is just one of several being considered by the boundary committee of the Commission on Stratigraphy. The question of synchronicity with the proposed Precambrian–Cambrian contact (China C marker) of Hsü *et al.*¹ cannot, therefore, be answered. Without supporting evidence for global catastrophism, such as coincidental anomalies in the platinoid elements, it is impossible to determine whether these isotope shifts reflect local or global processes.

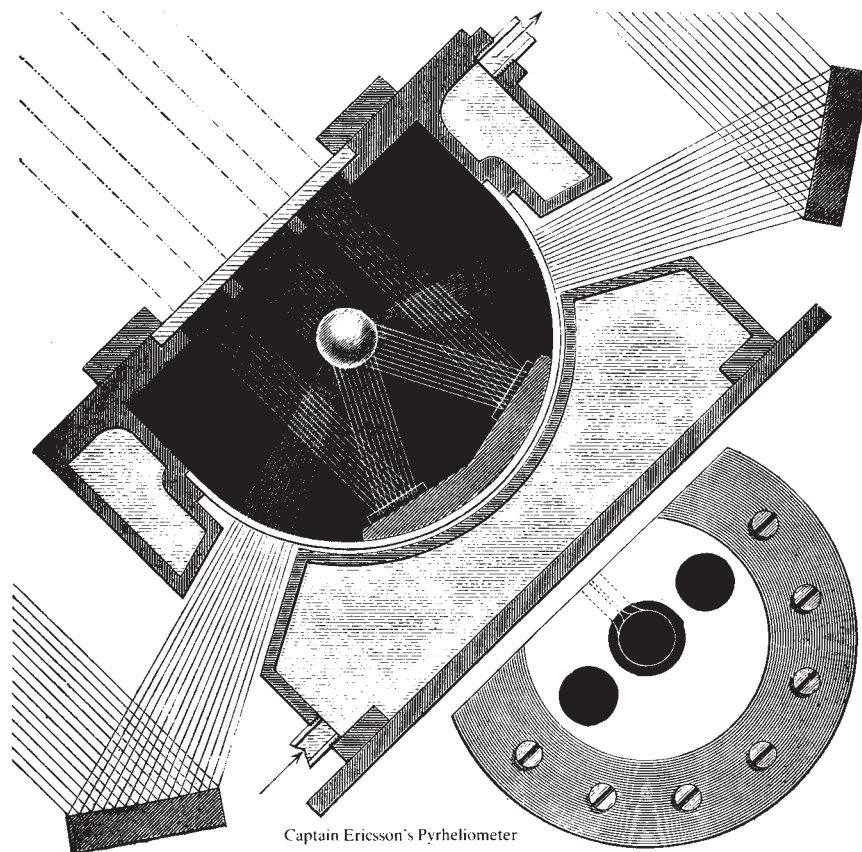
Although the sedimentation rate is poorly constrained, the time span represented by anomalous $\delta^{13}\text{C}$ values is probably large. Magaritz *et al.*⁵ are therefore dealing with relatively long-lived isotope changes that span tens of metres of section compared with the short-term effects that have been measured at mass-extinction boundaries^{1–4}. Any short-term perturbations which exist cannot be resolved because of the large sample spacing.

The problem with the use of phosphate deposits as indicators of a biomass bloom is that most of these deposits are poorly constrained in time, making correlations with boundary events difficult. Furthermore, the lack of a clear relationship between trends of short-term changes in carbon isotopes and phosphogenic episodes indicates that phosphate deposits do not reflect, at least on a global scale, periods of increased productivity. But major phosphate deposits do occur at the onset of episodes of vertical mixing after periods of oceanic stability⁷, which suggests that other factors, such as the ventilation of anoxic oceans, influenced the isotope composition of dissolved carbonate.

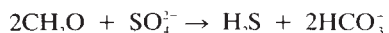
During the history of the Earth, the oceans were not always as well mixed or ventilated as they are today. Major periods during which the oceans were stratified with an upper oxygenated and lower anoxic water column have been identified for the Mesozoic⁸ and Palaeozoic⁹. Carbonates precipitated from the lower anoxic water column are enriched

100 years ago

from *Nature* 34, 249; 15 July 1886.



in ^{12}C because of the oxidation of organic matter by sulphate-reducing bacteria, as shown by the simplified reaction:



Because the amount of carbonate generated by this process is directly proportional to the fraction of sulphate reduced to sulphide, and as this fraction controls the $\delta^{34}\text{S}$ value (see figure legend) in the product sulphide, $\delta^{34}\text{S}$ values in pyrite provide a measure of the amount of organic carbon oxidized in anoxic basins. As shown in the figure for the Palaeozoic Selwyn Basin of northwestern Canada, $\delta^{34}\text{S}$ values increase during periods of ocean stratification until they approach or exceed those for coeval sulphate precipitated from the upper ventilated water column^{9,10}. Carbonates precipitated from the anoxic water column have $\delta^{13}\text{C}$ values as low as -10 parts per thousand.

On the adjacent Mackenzie Platform, carbonates deposited from the upper water column during the Ordovician and Silurian display $\delta^{13}\text{C}$ values more positive than those for average carbonates¹¹ (see figure). The ventilation of the oceans, by contrast, should result in a shift to more negative $\delta^{13}\text{C}$ values caused by the mixing of isotopically light carbon from the anoxic water column with surface waters. This relationship is illustrated for the Mackenzie Platform by a deflection to more negative values in carbonates of the

late Silurian (see figure).

The similarity of short-term carbon isotope trends in carbonates from the Mackenzie and Siberian platforms, both in the magnitude of the isotope shifts and in the polarity, indicates that biomass is not the only factor that influenced isotope ratios across the Vendian–Tommotian boundary. This is particularly true for stratigraphic horizons where synchronicity of events cannot be determined and where there is no supporting evidence for global catastrophism. More isotope and geochemical studies of carefully controlled sections sampled in detail across event boundaries are needed — only then will the processes controlling the fractionation of carbon isotopes be fully understood. □

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Palaeoecology

Surface water acidification

from Hans M. Seip

FEW topics have been the subject of so much recent debate as acid precipitation. Are there other causes for the surface water acidification and the dramatic loss of fish populations observed particularly in Scandinavia, but also in other parts of Europe and in North America? Palaeoecological studies, such as the one described on page 157 of this issue¹, support deposition of compounds of anthropogenic origin as the primary cause.

It is now generally accepted that the recent increase in acidity (H^+ concentration) of precipitation is caused by anthropogenic emissions of sulphur and nitrogen oxides. Recent surface-water acidification is also well documented. Although there is a reasonably good correlation in space and time between acid precipitation and water acidification, it is difficult to prove a causal relationship.

The main alternative hypothesis was originally forwarded by Rosenqvist², who believes that soil acidification caused by changes in land use is the most important cause of the recent water acidification. Although this view is not generally accepted, it has some support³ and there is a consensus on which processes to consider; the differences arise in their relative importance⁴.

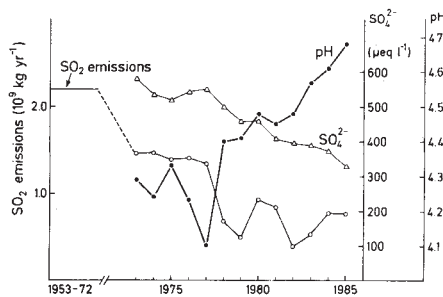
Because most of the precipitation will come into close contact with the terrestrial part of most catchments, acid soil is a prerequisite for acid water. Changes in the chemical properties of the soil, as well as changes in the hydrology⁵, affect the water chemistry. Several studies demonstrate soil acidification in Scandinavia and in Central Europe, which at least partly seems to be caused by acid deposition. Except for agricultural areas, estimation of H^+ fluxes to the soils supports the importance of acid deposition in acidification.

Water acidification depends not only on soil properties, but also critically on the concentration of 'mobile' anions, particularly sulphate, nitrate and chloride, that move easily through the terrestrial part of the system. Because a charge balance must exist in any solution, increased anion concentration will lead to increased cation concentration, including increases in H^+ and aluminium in runoff from acid soils. In most areas, the most important mobile anion seems to be sulphate; nitrate is mainly taken up by the vegetation but may affect water acidity, particularly during snow melt.

It is difficult to quantify the relative importance of acid deposition and other causes of water acidification. Simulation models indicate that increased sulphur

deposition may cause a considerable decrease in the pH of surface water while deposition may cause similar increases^{6,7}. These theoretical calculations are supported by observations around Sudbury (see figure), where the emissions have been dramatically reduced since 1972 and surface water quality is improving. Conversely, a historical approach used in three Norwegian studies did not reveal a relationship between land-use changes and loss of fish populations⁸.

Jones *et al.* combine a detailed palaeoecological study of vegetation changes from the last glaciation until the present day around a lake in Galloway, Scotland,



The decreasing emissions at Sudbury, Ontario, have resulted in decreasing sulphate concentrations and acidity in the Clearwater lake¹⁴.

with estimates of historical pH values using the diatoms in the lake sediments. Despite the rather dramatic changes in vegetation from a forest to an acid peatland, the pH of the lake remained surprisingly stable between 5.4 and 6.0 until the recent acidification started in about 1850. Although this study considers vegetation changes in particular detail, other palaeoecological results support the conclusion that acid deposition is a necessary condition for the recent acidification⁹⁻¹². For example, the pH of a lake in the Adirondack Mountains, United States, was about 5.7 during the period 1800–1950 despite major logging operations in the catchment. From about 1950 the inferred pH dropped to about 4.7 (ref. 12).

How reliable are these results? One complication is that the distribution of diatoms depends not only on pH, but also on other factors, such as the concentrations of humic substances and major ions in the water. The dependence on the content of humic substances has been used to infer changes from moderately acid humic water to more acid clear water in two Norwegian lakes, presumably because of acid deposition¹³. Seasonal variations in water acidity are often important and it is not clear how acid episodes are reflected in the diatom record. Despite some uncer-

tainties in interpretation, palaeoecological methods may be the best way to determine the importance of acid deposition.

Can we draw the general conclusion that water acidification does not occur in an area without acid deposition? The diatom studies usually give inferred pH values of more than 5 in pre-industrial times for lakes that are now acidified. This agrees with the observation that fresh water in 'clean' areas seldom has a pH less than 5.0. There are simply not enough anions to balance the charge of high concentrations of H^+ and aluminium ions. There are exceptions — highly coloured water may contain enough organic anions to make it more acid. But the recent acidification is not caused by increased concentration of organic compounds.

Sea-spray deposition with high concentrations of Cl^- and Na^+ may also cause acidification if sodium is retained and H^+ ions are released from the soil. But in a stable terrestrial system there will be no long-term accumulation of Na^+ and therefore only episodic acidification from sea-spray; in an aggrading system sodium may accumulate for longer periods.

Afforestation causes important changes in hydrology and soils, and several studies in the United Kingdom show more acid and aluminium-rich waters in afforested compared with non-forested areas in exposed regions. It is not clear whether a similar acidification would occur in areas with natural precipitation. The forest 'filters' the air and thus increases the deposition of both anthropogenic and natural compounds. Increased sulphate concentration in runoff may therefore be the main cause of acidification here.

In conclusion, there is at present little solid evidence for recent water acidification in areas not exposed to acid deposition. If occurring, it is likely to be found in areas with high sea-salt influence and recent vegetation changes such as afforestation, but the primary cause is evidently anthropogenic emissions of sulphur compounds. □

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Human gene cloning: the storm before the lull?

SIR—Hardly a week goes by without an article in *Nature* describing the cloning of a human DNA sequence. Yet it is barely nine years since the first cloning of a human gene sequence, chorionic somatomammotropin, was reported in *Nature*¹. Since then, some five hundred different human gene sequences plus many anonymous DNA segments, have been reported in a total of nearly 2,500 independent published articles² (as evidenced in *Gene Communications*, a quarterly updated newsletter obtainable from the authors on request). Such an exponential increase in knowledge accumulation is by no means unique. Examples of this phenomenon have included the literature explosions associated with cyclic AMP after the introduction of the second messenger hypothesis in 1958³ and the discovery and analysis of hereditary amino acidopathies after the advent of chromatographic methods⁴.

We calculate that were the present growth in the number of reports of cloned genes to be maintained, their number would overtake the total number of biological publications in 1992 and that of all scientific publications by 1994 (Fig. 1), still leaving the majority of the human genome untouched. However, this year the first signs of a plateau are becoming evident. It is interesting to speculate upon the various factors which might affect publication rates over the next few years.

A slow-down factor could be the finite size of the human genome (3×10^9 base pairs) — although the number of gene sequences which it contains, perhaps in excess of 100,000, is sufficiently large to ensure that there is a long way to go before any law of diminishing returns begins to operate. But given its limited and already stretched resources, the scientific community will sooner or later have to decide whether it can afford the not inconsiderable redundancy so characteristic of research efforts in this area.

This redundancy of effort is apparent from the number of independent reports

of the cloning of certain human DNA sequences. For example, the cloning of adenosine deaminase and of T-cell receptor α and β chain cDNAs have each been achieved eight times. There are 17 separate reports of a cloned *c-myc* genomic sequence and 33 independent reports of the cloning of the β -globin gene, although, in fairness, some of these are mutant alleles. While a certain degree of competition is to be expected and indeed welcomed in science, redundancy on this scale clearly calls for some more effective means of coordinating research efforts at the international level. It is, however, to be expected that no such let-up is likely in the hunt for those gene sequences deemed worthy of commercial exploitation. Presumably, strong competition will still characterize the drive to clone genes coding for proteins of economic and/or medical importance. Furthermore, an ever increasing number of laboratories are involving themselves directly or indirectly with cloning and mapping the human genome. The synthesis of molecular biology and human genetics seems able to attract an increasing number of adherents. How long the cloning fashion will be able to lure young researchers remains an open question, but for most laboratories, cloning is not an end in itself, but a means to an end, for example, functional analysis, spawning further booms in clone analysis.

One further factor which will affect publication rates is the future editorial policy of the eighty or so journals that at present publish these reports. Although to some extent offset by the likely future increase in journal numbers, the number of published cloning reports should tend to decline as a result of a probably tightening-up of standards and criteria for article acceptability. It is anticipated that authors will be expected to report on a larger number of sequences, a more complete sequence or to have undertaken in-depth structural or functional studies. Cloning data may in the foreseeable future have to be relegated to an information repository in the form of an electronic data base, a development which will be accelerated by

novel, more effective cloning, genome walking and sequencing strategies⁵. It remains to be seen whether the level of acceptance of such novel means of data storage and transfer is sufficient for any appreciable effect to become apparent in the near future.

Perhaps the rate of human gene cloning may even decline because people are simply getting bored with it. But it is also true that man's feeling of self-importance will probably not be satisfied until the last bit of his genome has been sequenced and filed somewhere.

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Immunotoxins to combat AIDS

SIR—Of the three therapeutic approaches to acquired immune deficiency syndrome (AIDS) suggested by Klatzmann and Montagnier¹, Singer and Shearer² have highlighted the possibility of attenuation of the CD4⁺ cells, which are the host for the virus associated with AIDS (HTLV-III/LAV), by infusing monoclonal anti-CD4 or anti-class II HLA reagents.

A more reasonable approach, we suggest, would be to use immunotoxins³ — conjugates of a specific antibody and a plant or bacterial toxin — directed specifically to the CD4⁺ cells. The toxic moiety is a ribosome inactivating protein (RIP)⁴, either a single A chain or both an A chain and a B chain (which is a monovalent lectin). Immunotoxins containing A-chain RIPs have a definite advantage over the two-chain RIPs as they can be made absolutely target specific⁵. There is now good evidence that only one molecule of the enzymatic, toxic A chain entering the cytoplasm of a cell is sufficient to bring about the cell's destruction by totally inactivating its protein-synthesizing machinery⁶. Immunotoxins offer the advantage of providing both specificity of targeting through the antibody moiety and extremely effective inhibition of messenger RNA translation (which is increased by orders of magnitude by the product of the *tat-III* gene of HTLV-III/LAV in infected cells⁷) by virtue of the toxophore moiety.

By subverting the stratagem used by the virus for its replication, the administration

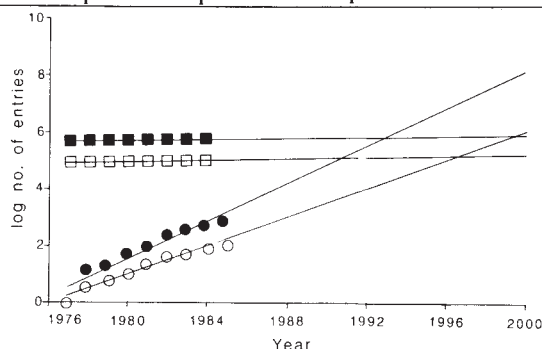


Fig. 1 Exponential growth of publications per annum on cloned human DNA sequences: all reports (filled circles) and first reports on protein coding sequences (open circles). For comparison the number of publications covered by *Biological Abstracts* (open squares) and *Science Citation Index* (filled squares) are given.

of immunotoxins would greatly diminish the number of virus particles released, over a period of time, into the circulation following the destruction of CD4⁺ cells. As a consequence of this reduced number of viral particles thus released, they could probably be neutralized effectively by the antibodies already present in the patient's blood. On the other hand, infusion of anti-CD4 antibody alone (which by itself cannot attenuate the viral population) would release far greater numbers of viral particles. As a result, the neutralizing capacity of the circulating antibodies may be considerably reduced. The injection of anti-viral antibodies to neutralize the virus would then be required. Because of the antigenic drift⁸ noted with this virus, ex-traneous infusion of anti-HTLV-III/LAV antibodies may not be particularly helpful in neutralizing the large pool of HTLV-III/LAV released by treatment with anti-CD4 antibodies.

Thus the administration of immunotoxins in minuscule amounts (as RIPs act in a catalytic manner) might forestall the spread of AIDS virus. Moreover, as only a small proportion of CD4⁺ cells are infected by the virus¹ and as fresh peripheral blood lymphocytes of AIDS patients do not show the presence of viral DNA, there will probably be no necessity for immun-enhancing therapy with immunotoxins.

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Earthquake prediction and electric signals

SIR—In a comment in *News and Views*¹ P.W. Burton discussed our publications in *Tectonophysics*² on the prediction of the epicentre and the magnitude of earthquakes within a time window of 7 to 115 hours, based on the monitoring of the simultaneous changes of the electric field of the Earth observed at a number of sites. Burton expressed doubts about the claimed connections between earthquakes and the precursor signals, especially during periods of high seismicity. Of course, there is a high probability of an earthquake occurring somewhere within the time window, but we have isolated a large number of events in both time and seismic region.

A recent case, with an exceedingly small probability of having been predicted by chance, illustrates our point. On 17

December 1985, we presented a prediction to the session of the Greek Special Committee on Earthquake Prediction, which was recently established by the Greek Ministry of Public Works and consists of geologists, seismologists and physicists. The electric signals allowed two solutions, one for an event of magnitude 4.8 in the sea to the south of Kalamata (southern continental Greece) and the other for a 5.2 mag event on the coast of Asia Minor near the island of Samos. Forty hours later a 5.2 mag event occurred within 150 km of the site expected from the second solution. As no earthquake with $M > 4.7$ had occurred within the 75,000-km² area 36.7–40.0°N and 25.0–27.5°E for at least the previous 14 months, the time-probability is smaller than 10⁻². Obviously the probability of simultaneously predicting the time, epicentre and magnitude is appreciably smaller.

Another recent case is the following: On 29 March 1986 a 6.1 mag event occurred at 38.3°N–25.3°E. Four days earlier the government had been officially informed of an impending 6.1 mag earthquake. The error in the prediction of the epicentre was less than 50 km.

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The stability of zoological nomenclature

SIR—As one of those cast by Erzinclioglu and Unwin (*Nature* **320**, 687; 1986) as “having a legalistic turn of mind”, I would like to protest the spirit, as well as the content, of their letter which was intended to detail the inadequacies of the *International Code of Zoological Nomenclature* and to protest the “tyranny of the Commission”.

First, the writers do not seem to understand the meaning of stability in the sense of the code. Stability has never been taken to mean that a name shall remain unchanged and unchangeable: the preamble of the *Code* states that, “All its [Code] provisions and recommendations are subservient to these ends [promoting stability and universality in the scientific names of animals] and none restricts the freedom of taxonomic thought or action.” Further, the plenary powers of the Commission, defined in Article 79, outline the responsibility of the body to maintain stability and specifically refer to the suppression of a senior homonym as a potential option in assuring stability.

With regard to a few specific points raised by Erzinclioglu and Unwin, the

Code has, for several editions, made a clear distinction between the endings of names and the name of the taxon. Thus, changing the ending of a species name to agree with that of the genus in no way constitutes a change of names.

A similar observation, of course, applies to the authors' argument regarding elevation of subfamily groups to the family level. The reasoning behind the rules governing change of rank within the family-group seems altogether clear and I cannot imagine such a change could cause confusion.

In their lengthy summary of the change of species names for the medically important African fly *Auchmeromyia luteola*, or perhaps I should say *A. senegalensis*, the writers do not seem to be offended by the change in generic placement, which presumably occurred before their entry into the world of nomenclature, but only by the discovery of an obscure senior homonym and subsequent name replacement. Their irritation is misplaced and might better have been directed at the author(s) of *Catalogue of the Diptera*, who chose to offer a replacement name rather than to maintain the well-known name and refer the matter to the Commission. This option has existed for years and is the rational way to resolve this kind of problem and assure stability.

Erzinclioglu and Unwin seem particularly confused regarding the relationship between the rules of nomenclature and the classification of organisms. Adherence to the principles of cladism or, for that matter, “traditional” systematics have no bearing whatsoever on the value of the *Code*.

Finally, those of us who “mindlessly” adhere to the *Code* resent the notion that anarchy is the only sane alternative to felt weaknesses in the rules. There is, and always has been, the alternative of petition for change in the document. In my opinion the present *Code* constitutes an improvement, in some areas, over the previous edition. There remain very real problems regarding application of the rules to collective groups and ichnotaxa — problems that will probably never be resolved to everyone's satisfaction — but there is a mechanism within the rules to allow change to be proposed.

I find it ludicrous to consider the Commission as a group of hooded tyrants whose purpose it is to impose their arbitrary will upon the scientific community, from which, of course, they were elected. It may come as some consolation to Erzinclioglu and Unwin to note that by the act of expressing their interest in nomenclature, they have qualified themselves for nomination to election to the Commission.

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The path to solid success

R. V. Jones

A Life in Science. By Sir Nevill Mott. *Taylor & Francis*: 1986. Pp. 198. £15, \$30.

NEVILL Mott's concise autobiography shows how fortunate he was in his parents. They had both been research students at Cambridge under J.J. Thomson, and throughout their lives he could share with them his professional experiences in a happy manner rarely granted to a physicist. They could thus take informed pleasure in his first success in research, the prediction in 1929 — on the basis of the new quantum mechanics — of the way in which α -particles would be scattered by helium nuclei.

They could also enjoy pen-pictures of life in Copenhagen with Niels Bohr, in Göttingen in the earliest days of quantum mechanics, and back in Cambridge with Rutherford and Dirac. Writing of his Cambridge and Copenhagen periods, Mott twice uses the phrase "sink or swim" to describe the then prevailing atmosphere in which research students were expected to fend for themselves. This will ring true to those of us who entered physics before 1939.

His time as a student ended in 1929 with appointment to a lectureship with W.L. Bragg in Manchester. He held this for only a year, before an invitation from A.M. Tyndall took him to Bristol as professor of theoretical physics at the age of 28. Protected from administration by Tyndall, one of the wisest of laboratory heads, Mott pursued his developing interests in solid state physics; and over the next dozen years he became senior author of three notable books, "Mott and Massey" *Theory of Atomic Collisions*, "Mott and Jones" *Theory and Properties of Metals and Alloys* and "Mott and Gurney" *Electronic Processes in Ionic Crystals*. He was only 31 when elected to the Royal Society — "Nowhere near a record", he tells us, for "Paul Dirac was elected when he was 28".

The Second World War brought Mott into contact with the Army, first at Anti-Aircraft Command, then at the Army Operational Research Group at Peter-sham and finally at Fort Halstead as Superintendent of Mathematical Research in Armaments. Those who knew him before 1939 were struck by the way the war brought him out of his shell. As for many of us, he was now working with soldiers and grappling with practical problems of the utmost importance. I remember his contribution to the construction of reflecting screens of chicken wire for radar aerials: if a particular mesh would let 10 per cent of the energy leak backwards,

then halving the pitch of the mesh would roughly halve the leakage: but if instead of using extra wire in the closer mesh, the same amount were woven into a second screen placed behind the first, then each screen would only let through 10 per cent of the radiation incident upon it, and so the double screen would let through only one per cent. But while Robert Watson-Watt could well have appreciated the elegance of this idea, I cannot imagine him being so happy with Mott's giving the credit, as he does, to Edward Appleton for the use of pulse methods in radar.

The experience at AA Command gave Mott "a deep respect for the professional soldier". At the same time the war led him to realize the importance of industry to Britain, and the benefits that could ensue

taken not by him but by the body of fellows. Tizard had experienced the same thing at Magdalen in Oxford where, as the *Dictionary of National Biography* records, he found it difficult "to pilot controversial issues through a very varied and independent-minded body of fellows". "Looking back", Mott writes, "on both my election and my resignation, it seems like one of Snow's novels; we used to say that Snow 'didn't know the half of it'".

When he resigned from Caius in 1966 Mott still had another five years in the Cavendish chair, where he devoted much effort to teaching, both in the university and in schools. He chaired the physics committee of the Nuffield Scheme, where the main idea was "learning by doing". But, he writes,

I would have hated to do it this way. To me the laws of physics are to be approached through mathematics, and their validity and beauty become clear when expressed in mathematical form. My hero was Clerk Maxwell rather than Faraday....

He also had reservations, although he did his best for the scheme, over the Treve-



Nobel quintet — (left to right) Philip W. Anderson, W.H. Brattain, Nevill Mott, John Bardeen and J.H. Van Vleck. Anderson, Mott and Van Vleck were joint Laureates in 1977, and Brattain and Bardeen (with William Shockley) in 1956. Bardeen was again a winner in 1972, with Leon Cooper and J. Robert Schrieffer.

if university science were more closely linked to it. This was one factor that led him to return to Bristol rather than accept a chair he was offered in Cambridge.

The post-war years in Bristol scintillated with success. Mott succeeded Tyndall as head in 1948, and was surrounded by a galaxy of talent that included C.F. Powell, C.R. Burch, P.H. Fowler, F.C. Frank and J.W. Mitchell. But when, in an act of self-sacrifice, W.L. Bragg left the Cavendish chair in 1953 to restore the fortunes of the Royal Institution, Mott accepted appointment as his successor — just too late for his mother to be told of this "overwhelming honour".

In 1959 Mott also became Master of Caius College, Cambridge: surprisingly, it had become fairly common in Cambridge for an individual to be head of a college, as well as of a large laboratory. Mott found the two jobs very different. In the laboratory, after listening to opinions, he took the key decisions himself: in college he was expected to abide by decisions

lyan scholarships initiated by Kurt Hahn: "It was dangerously near 'character rather than intellect' which was of course anathema to me". He was happiest with his chairmanship of the Royal Society's education committee, writing a report in 1979 on the needs of talented children. The report concluded "that mixed ability teaching could be successful only with exceptionally able teachers"; instead "our working party believed that as far as possible talented children should advance at their own pace, and with this in view favoured 'setting' in science and mathematics from 13 years onwards".

Relinquishing the mastership of Caius gave Mott more time for research, and his interest was aroused by the properties of non-crystalline semi-conductors. His contributions to the theory, which have continued after his retirement from the Cavendish in 1971 to the present day, were recognized by the award of a Nobel Prize in 1977. So while advances in theoretical physics are nearly always made by

young men, Mott's best-rewarded work has been done, most remarkably, in retirement — an exemplary encouragement to us all.

As for his method of creative reasoning, some clue may be gained from Smoluchowski's retrospective comment on the early days of solid state physics: "Mott... and his colleagues horrified us by their 'simple' visualizable and seemingly uncomplicated models and mathematics". So, with his models such as "swollen atoms", Mott is in a tradition expressed by H.G.J. Moseley in 1914: "the French point of view is essentially different from the English. Where we try to find models and analogies, they are quite content with laws". By his practice, then, Mott has a foot in one camp, and by his professed predilection for laws, also a foot in the other. Bestriding both, his is one of the most sustained lives of success in physics, recognized by numerous medals and 27 honorary doctorates.

A Life in Science is written with great economy, throwing laconic sidelights on many issues into which Mott's pursuit of science has drawn him. "Kindness with firmness" is a pervading trait, the former being exemplified in his remark that "The greatest pleasure in life is putting others on the way to success", the latter in stopping the project for a linear accelerator in Cambridge in one of his first acts as Cavendish professor.

In the deepening attention that many in science are giving to religion, Mott's own personal statements on the matter will be appreciated. In 1929 he wrote to his fiancée, "If you *wanted* to become a Roman Catholic, I should want to die. The trouble is that I can't think about the institution because it fills me with absolute physical disgust...". While this pristine view has been much mellowed by age and by an appreciation of the services performed by many devoted Catholics, "something of my antagonism remains". But Mott feels no such antagonism for the Anglican Church: indeed in retirement he has been baptized and confirmed as a member, and he devotes a whole chapter of the book to religion. He regularly attends church

finding it helpful to worship God in company with other people. If some things that are repeated in the Creed do not correspond with what I believe, such as 'born of the Virgin Mary', I accept what is said because to me the Christian religion is the sum of the beliefs of Christians throughout the ages, not only those of our present generation.

Each man's religion is a matter for himself, and we can only be grateful to one who has spent a long life so eminently in science for exposing so frankly his personal conviction. □

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Getting the picture to tell the story

P.W. Hawkes

Image Analysis: Principles & Practice. Joyce-Loebl:1986. Pp.250. Pbk £35, \$50. *

THERE are four grand themes of digital image processing: acquisition and coding; enhancement; restoration; and analysis, including pattern recognition. This last activity differs from the others in that the result is not a better image or one that is transformed in some way, but information about its contents. The information often comes in the form of a histogram or tables of data. In a karyogram, for example, the properties of the various chromosomes present might be listed; in quality control in a manufacturing process, certain dimensions might be monitored and the process interrupted if these exceeded preset limits; in aerial cartography, areas of forest and field, river and lake, factories and dwellings might be recognized, measured and their relative separations characterized in some way.

There have been many books on image analysis but none quite like this one — in

* Available from Rosalyn Kell, Joyce-Loebl, Marquisway, Team Valley, Gateshead, Tyne & Wear NE11 0QW, UK, or Bill Burnip, Joyce-Loebl, c/o Nikon Inc., 623 Stewart Avenue, Garden City, New York 11530, USA.

simple language, and with ample illustration, it enables the reader to use a commercial image analyser to the best advantage. The coverage is broad but is not correspondingly shallow, though the mathematical foundations of the various operations are rarely described. The text covers acquisition and digitization, but it will be used principally for its chapters on image processing, detection and measurement techniques. These introduce the reader to the ways of locating specific structures, of amending images by erosion or dilation (*sic*), of identifying and coding boundaries, and of characterizing shape, among many related topics. Subsequent chapters deal with colour images, computing (almost too brief to be useful) and a number of case studies. Each chapter ends with a bold-face summary; these read more like collections of great thoughts than scientific abstracts: "classification is a useful technique, but can become complex if many different kinds of measurements are used" gives an idea of their profundity.

Excusably, the anonymous authors had the Joyce-Loebl analyser in mind, but the book is not unduly partisan and gives an exceedingly clear and easily assimilated account of a great deal of disparate material. There is no better book for anyone involved with these machines who would be daunted by a more mathematical account. □

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Disordered thoughts

P.W. Atkins

The Unfinished Universe. By Louise B. Young. Simon & Schuster:1986. Pp.239. \$17.95.

THE argument of this book is that a great creative process is taking place within the Universe, with us as witnesses and participants, and that matter has an innate tendency to achieve greater complexity. *Form* is emerging like a butterfly from a chrysalis, and the process that began when quarks coalesced into protons is now moving towards its culmination. The argument depends on a specific rejection of the view that the Universe is running down into a state of disorder.

The case in favour of this formative tendency is passionately argued, and it is plain from the style of the exposition that Mrs Young is deeply in love with the world and with her vision of science, and longs to share her wonder and awe. Such books should be approached with a special cast of mind, for it is important not to allow the style to obscure the content. This is espe-

cially true of one wrapped in a jacket bearing messages from three highly respected scientists, one of whom claims that the book releases us from the stifling imprisonment of the Second Law of thermodynamics, another that Mrs Young's view that a creative process is taking place "is an idea... of great importance" and a third who finds it a strangely beautiful guide.

I feel outnumbered, for I find it none of these things. Passion there certainly is, and I can see why some might find the prose "lovely". I will disregard the little slips that don't matter very much (such as ascribing seven electrons to chlorine and the heat of the Earth to thermonuclear reactions in its core). I will also largely ignore (but it set my antennae waving) the author's rejection of the concept of force and its replacement by some kind of vague, non-quantified, tendency of particles to congregate into more complex form (p.41). I will concentrate instead on the central idea, that the Second Law cannot fully account for the phenomena of the world, and particularly for the phenomenon of life. Mrs Young admits (p.120) that the trend postulated by the Second Law is the opposite of the one she proposes, and therefore her thesis depends on

her ability to demonstrate the Law's invalidity and incompleteness.

I can see why Mrs Young thinks it inadequate: it is because she appears not to have understood it. Chapter 6 is a travesty of thermodynamics. In the first place, a waterfall (p.119) is an excellent example of a process on which, as expressed, the Law is silent. Secondly, when the Law is first presented (p.119) it is stated in terms of the tendency of the entropy of a system to increase, without it being emphasized that the system must be isolated. A partial recovery is made three pages later when Mrs Young reminds us that physicists have "explained" (her quotation marks) the apparent spontaneous local emergence of order by insisting that the Second Law refers to a totally closed (by which she should mean isolated) system. However, she rejects this "explanation" by remarking (p.123) that it is not possible to ascribe numerical values to "the different levels of complexity" of a system, which I take to be the denial of the view that the entropy change in a system can be expressed quantitatively. That of course is silly: huge tables of thermodynamic data exist and other data can result from well-defined experiments. One of the main objects of statistical thermodynamics is to do this very calculation.

Slippery language erects false tests. In what should be a technical account of entropy and its calculation, the author asks (p.124) "how can we place a value on the creation of the first living thing?" as a paraphrase of "what is the change of entropy of the system?". Moreover, in a book on the application of thermodynamics to life, surely one should expect to find a discussion of the behaviour of open systems, dissipative structures and entropy production in states far from equilibrium?

Far from being a release from the stifling imprisonment of the Second Law, the book is an essay based on misunderstanding. Far from being a strangely beautiful guide it is a snare for those with a passion to know but not the ability to judge. Far from being important enough to be published and await its time (p.227), the central thesis is nonsense. I am sorry to be so harsh, for I can sympathize with the author's passion. □

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New in paperback

- *Time Frames: The Rethinking of Darwinian Evolution and the Theory of Punctuated Equilibria* by Niles Eldredge. Publisher is Touchstone (Simon & Schuster), price is \$8.95. For review see *Nature* **316**, 683 (1985).
- *Imagery in Scientific Thought* by Arthur I. Miller. Publisher is MIT Press, price is \$8.95, £8.95. For review see *Nature* **314**, 689 (1985).

Down to physicists

Peter J. Smith

Continuum Theories in Solid Earth Physics. Edited by R. Teisseyre. Elsevier:1986. Pp.566. Dfl. 290, \$107.50.

THE American geologist M. King Hubbert was wont to startle his audiences with the proposition that as science advances it becomes not more complex but simpler. This is, of course, one of those intellectual teasers that is both true and false but more false than true. What King Hubbert had in mind was the plethora of apparently disparate observations and unrelated hypotheses which, once plate tectonics had been discovered, could be summarized as a paradigm in half a page. He had a nice philosophical point, but one that ignores the fact that working Earth scientists are not primarily philosophers. Students and researchers, forced to look behind the simplifying mega-patterns of the Earth, are all too aware not only of the increasing complexity but also of the growing burden of knowledge represented by ever more detailed observations, hypotheses and interpretations.

There is, moreover, a quite different sense in which King Hubbert's dictum is suspect. His exemplifier, plate tectonics, is essentially a theory of *what* happens rather than of *how* or *why*. It has been clear for some time now that the latter questions can only be addressed in terms of the solid-state physics of the Earth's interior, a development that raises complexity to bewildering heights for those versed only in conventional geological methods. *Continuum Theories in Solid Earth Physics*, which originated in Poland, proves the point with a vengeance. About 80 per cent mathematical and requiring a good working knowledge of tensor analysis applied to continuous media, the book demonstrates just how "non-geological" the fundamental problems of Earth behaviour really are.

The topics on the agenda here are the theory of Somigliana dislocations and its application to earthquake mechanics and aseismic creep; fracture theory and its relevance to seismology; the mechanics of continuous media with special reference to seismology; the behaviour of porous media; the theory of thermal stress in the Earth's interior; thermal convection; the nature of electromechanical and magnetohydrodynamic processes in the Earth's core as the source of the geomagnetic field. In each case, although the emphasis is on mathematical development, due attention is paid to the need to relate theory to the results of observation and experiment (not always a feature of books of this kind) and for the most part there is sufficient

description to enable the non-mathematically inclined to get at least the gist of what is going on. It is interesting, too, to note that the Poles are far more familiar with what goes on in the West (and Japan) than are Western scientists with developments in Poland. As a result, there is little evidence here of a "Polish view" of Earth physics and thus, in contrast to many treatises emanating from the Soviet Union, no false impression of the subject having originated and developed quite separately in the East.

Whilst this commendable internationalism gives point to the publication of the book in Poland, it is less easy to see the merit in making a somewhat coals-to-Newcastle translation into English. The very lack of parochialism has the ironic consequence that there is little, if anything, here to be learned by Western Earth scientists. Nor, even if there were no comparable texts already in the West, would the volume be a suitable text for the uninitiated. As D.L. Turcotte and G. Schubert so ably showed in *Geodynamics: Applications of Continuum Physics to Geological Problems* (Wiley, 1982), it is perfectly possible to teach Earth physics without tensors and get through to a wider audience into the bargain. □

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How important is natural selection?

J.S. Jones

Natural Selection in the Wild. By John A. Endler. Princeton University Press:1986. Pp.336. Hbk \$40, £26.65; pbk \$13.95, £9.30.

THE most influential evolutionary book of the past ten years is, without doubt, Motoo Kimura's *Neutral Theory of Molecular Evolution*, and of the decade before that R.C. Lewontin's *The Genetic Basis of Evolutionary Change*. Lewontin pointed out the difficulties of explaining molecular polymorphism using traditional models of natural selection acting at single loci, while Kimura argued cogently that the most important force in controlling the evolutionary fate of most newly arising mutations is not selection but random genetic drift. This relegation of natural selection to the role of evolution's policeman, who acts mainly to remove alleles deviating from an acceptable norm and ignores the vast majority of genes in populations, is now central to large parts of theoretical population genetics, to molecular evolution and to the reconstruction of phylogenies. John Endler's impressive book attempts to restore natural selection to its traditional place as the driving force of evolution. It may become an evolutionary classic of the next decade.

As Endler points out, the term "natural selection" has meant very different things to different people. After wandering briefly in the philosophical fog which is thicker in evolutionary biology than in any science apart from psychology, he produces a definition of selection close to the Darwinian one — variation, inheritance and genetic differences in the chances of reproduction — or even to that of Oscar Wilde, that "nothing succeeds like excess". Natural selection does not necessarily lead to evolution, and it includes many biological processes apart from differential mortality. Indeed, a survey of experiments on selection in nature suggests that inherited differences in survival are less important than are those associated with mating ability, fertility or fecundity.

There have been numerous attempts to measure selection in the wild. Many of these have failed because of poor experimental design, and Endler suggests a number of ways in which selection might acceptably be demonstrated in natural populations. He discusses ten kinds of information that can identify selection, from simple correlations of gene frequency with environment, to comparisons of the same genes in related species, and

to demographic studies and experimental perturbation of populations in nature. The road to detecting selection has many pitfalls. Endler finds 25 reasons why we might fail to detect it when it does exist but, comfortably, only 21 ways in which selection might be identified when in fact it is not operating. His extensive analysis of work on natural selection in the wild suggests that strong selection on morphological characters and polymorphic alleles — selection of the intensity used by animal and plant breeders — is commonplace in nature, and that the general assumption that differences in fitness of more than about 10 per cent are rare is simply incorrect. It is nevertheless rather depressing to learn that there is as yet no case in which natural selection acting over an organism's whole lifetime has been measured. It is also true that if size and shape are controlled by many genes of individually small effect, then strong selection on morphological characters may still allow genetic drift to determine the evolutionary fate of most mutations at such loci. This distinction between natural selection on the phenotype and the genotype may yet bridge the gap between those who emphasize the importance of random change in evolution and those who hold the views so eloquently put forward in this book.

Endler himself is sanguine that "as our knowledge . . . increases, perhaps natural selection will become easily detectable at the biochemical and even the molecular level". Although ecological genetics has a

long way to go before this hope will be fulfilled, it is nevertheless true that a hypothesis that selection acts on a particular system is, like the search for the North West Passage, intrinsically productive even if it is wrong. All too often great pyramids of theory have been balanced on a mere assumption that selection is unimportant: the neutral theory has been much more of a stimulus to theoretical than to experimental studies of evolution. Theory frequently asks us to measure the unmeasurable, and experiments on the genetics of populations in nature are often designed in such a way as to produce results which cannot be interpreted. What is needed is a joint approach to the problem of the importance of selection in evolution: to use one of the quotations from Dr Johnson which preface each chapter, there is nothing

more pleasant, or more instructive, than to compare experience with expectation, or to register from time to time the difference between idea and reality. It is by this kind of observation that we grow daily less liable to be disappointed.

Natural Selection in the Wild is a unique blend of ideas about evolution set against the reality of studying it in nature. All evolutionists should read the book: it will disappoint none of them, whatever their preconceptions about the importance of natural selection. □

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THE INVAGINATE GASTRULA AND THE PLANULA

A giddy little *Gastrula*, gyrating round and round,
Was thought to show the way we got our enteron profound:
A little whirlpool in its wake maintained a tasty store,
A pocket sank to lodge it all, and left a blastopore.

As a larval epigram this description earns a prize,
But as sketching adult ancestry can only win surprise,
And when you note all early orders fixed upon the rocks,
You feel a slight embarrassment, the first of many shocks.

The foremost inconsistency is the simple, solid fact
That the Hydrozoan larva, the mouthless *Planula*, is packed
With a jumbled mass of gastric cells that drop in, slow or fast,
And show no slightest cavity till the larval stage has passed.

The larva then becomes attached, and shortly stands erect,
Nourished by the yolk stores the inner cells eject:
Their shrinkage leaves a growing space, the early enteron,
Round which a layer of cells remains, and lines the outer one.

Some tentacles are sprouted then, say 2, then 4 and 8,
And not till all is ready does the mouth break through quite late.
If mouth and gut arose at first from one invagination,
What roundabout procedure's here! What needless complication!

Invagination surely is a thing of later date—
Procedure speeded up to suit the embryonic state:
The cells as loose irregulars build up the lower grades,
And yield but slowly, step by step, to organised brigades.

Reason in rhyme — this poem is an attack by Walter Garstang on Haeckel and his followers, who, in support of their "biogenetic law", invented an ancestor for all metazoans (the *Gastrea* theory) and claimed to be able to trace the evolution of the gut from a dimple in the surface of the primordial metazoan. It is reproduced from a new paperback reprint of Garstang's *Larval Forms and Other Zoological Verses*, published by University of Chicago Press. Price is \$5.95, £4.95.

The mystery of declining tooth decay

from Mark Diesendorf

Large temporal reductions in tooth decay, which cannot be attributed to fluoridation, have been observed in both unfluoridated and fluoridated areas of at least eight developed countries over the past thirty years. It is now time for a scientific re-examination of the alleged enormous benefits of fluoridation.

FLUORIDATION consists of raising the concentration of the fluoride ion F^- in water supplies to about 1 part per million (p.p.m.) with the aim of reducing dental caries (tooth decay) in children. In fluoridated areas, there are now many longitudinal (temporal) studies which record large reductions in the incidence of caries¹. The results of these and of fixed time surveys have led to the 'fluoridation hypothesis', namely that the principal cause of these reductions is fluoridation.

Until the early 1980s, there had been comparatively few longitudinal studies of caries in unfluoridated communities. Only a small minority of the studies in fluoridated areas had regularly examined control populations, and there seemed to be little motivation to study other unfluoridated communities. But during the period 1979-81, especially in western Europe where there is little fluoridation, a number of dental examinations were made and compared with surveys carried out a decade or so before. It soon became clear that large reductions in caries had been

occurring in unfluoridated areas (see below). The magnitudes of these reductions are generally comparable with those observed in fluoridated areas over similar periods of time.

In this article, these reductions are reviewed and attention is also drawn to a second category of caries reduction which cannot be explained by fluoridation. This category is observed in children described by proponents of fluoridation as having been 'optimally exposed', that is, children who have received water fluoridated at about 1 p.p.m. from birth. The observation is that caries is declining with time in 'optimally exposed' children of a given age. In some cases, the magnitudes of these reductions are much greater in percentage terms than the earlier reductions in the same area which had been attributed to fluoridation.

The problem of explaining the two categories of reduction goes well beyond the field of dentistry: contributions from nutritionists, immunologists, bacteriologists, epidemiologists and mathematical

statisticians, amongst others, may be required.

Caries in unfluoridated areas

Table 1 lists over 20 studies which report substantial temporal reductions in caries in children's permanent teeth in unfluoridated areas of the developed world. In many of these cases, the magnitudes of these reductions are comparable with those observed in fluoridated areas and attributed to fluoridation.

Several of these studies give clues as to factors which are unlikely to be the main causes of the reductions. A comparison of the 1954 and 1977 dental health surveys in Brisbane^{2,3} indicates to a reduction of about 50% in caries, as measured by the number of decayed, missing and filled permanent teeth (DMFT) per child and averaged over the age groups, in the 23-year period. The 1977 survey distinguished between children who took fluoride tablets regularly, irregularly or not at all. Although there were differences in caries incidences between the three categories (which could reflect factors unrelated to fluoride levels), even the "no tablet" group had on average 40% less caries experience than that recorded in 1954. So fluoride tablets were not the principal cause of the reductions observed in Brisbane.

The first Sydney study⁴ showed that children with "naturally sound" teeth increased from 3.8% in 1961 to 20.2% in 1967 and 28% in 1972. The paper, which was titled enthusiastically "The Dental Health Revolution", was originally used widely to promote fluoridation in Australia. The authors stated that: "Almost certainly, the availability of fluoride both in tablet form and delivered through town water supplies has been the predominant factor. . . . These very large reductions represent a modern triumph of preventive health care"⁴. Yet the major proportion of the reported improvement had already occurred before Sydney was fluoridated in 1968. Moreover, no evidence was presented that fluoride tablets were widely used in the 1960s. Fluoride toothpaste was only introduced into Australia in 1967³. Although the index "naturally sound" teeth is unsuitable for more detailed

Table 1 Studies reporting large reductions in dental caries in unfluoridated areas

Location		Years surveyed	References
Australia	Brisbane	1954, '77	2, 3
	Sydney	1961, '63, '67	4
Denmark	Various towns	1972, '79	53
Holland	The Hague	1969, '72, '75, '78	38
	Various towns	1965, '80	11
New Zealand	Auckland (parts)	1966, '74, '81	12
Norway	Various towns	1970, '80	54
Sweden	Various towns	1973, '78, '81	39
	North Sweden	1967, '77	55
United Kingdom	Bristol	1970, '79	56
	Bristol	1973, '79	56
	Devon	1971, '81	37
	Gloucestershire	Annually from 1964	37*
	Isle of Wight	1971, '80	57
	North-West England	1969, '80	58
	Scotland	1970, '80	59
	Shropshire	1970, '80	10
	Somerset	1975-79 annually	60
United States	Somerset	1963-79	61
	Dedham, Mass.	1958, '74	40
	Norwood, Mass.	1958, '72, '78	40
	Massachusetts: sample of schools	1951, '81	41
	Ohio	1972, '78	62

* Unpublished communication from J. Tee (1980), Area Dental Officer, Gloucestershire, to R. J. Anderson *et al.*³⁷

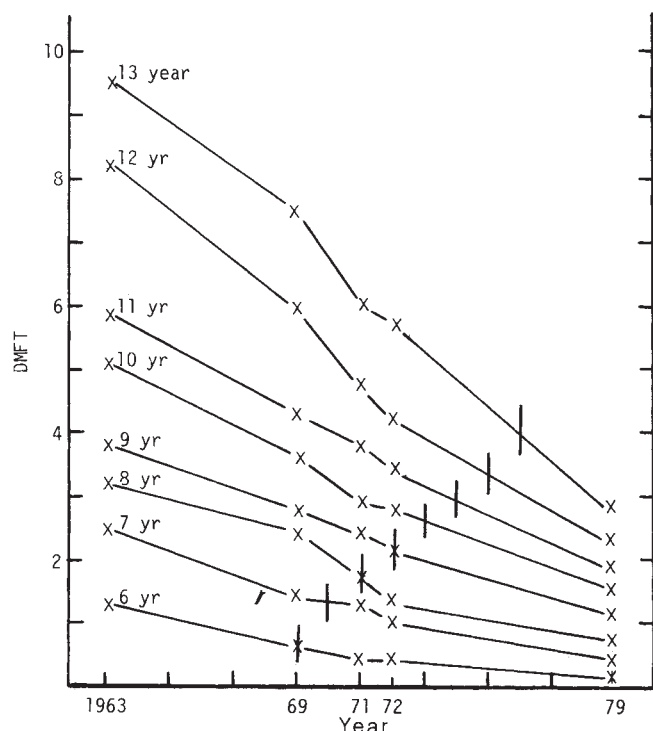


Fig. 1 Decline in caries, as measured by DMFT, in Tamworth, Australia, for children in age groups 6 years to 13 years. Data compiled from refs 14, 15. The vertical line cutting graph for each age group denotes year at which maximum possible benefit from fluoridation was reached. Tamworth was fluoridated in 1963.

studies which distinguish decayed, missing and filled teeth, the populations examined were very large (over 9,000 children at each examination) and the results clear-cut.

A second Sydney study⁵ used the DMFT index, but was irrelevant for establishing any link with fluoridation, since it reported only on examinations in 1963 and 1982, but not around 1968 when Sydney was fluoridated. As in several other fluoridation studies, the key data were either not collected or not reported⁶. Although the two Sydney papers have an author in common (James S. Lawson, a senior officer of the New South Wales Health Commission), the second paper does not even cite the first. This suggests that, once it became clear that the first Sydney study contained evidence unfavourable to fluoridation, it was a source of embarrassment to some fluoridation proponents who are apparently trying to denigrate it.

However, independent confirmation of the large reductions in caries before fluoridation reported in the first Sydney study⁴ is readily obtained by comparing the results of two surveys^{7,8} separated by 20 years by Barnard. These surveys showed that the mean DIMF index ('I' denotes a permanent tooth which cannot be restored) for school children aged 13 and 14 declined from 11.0 in 1954–55 to 6.0 in 1972. The four years from 1968, when fluoridation commenced in Sydney, to

1972, would not have contributed significantly to the decline in caries prevalence in this age group⁹.

The authors of one of the British studies¹⁰ cited in Table 1 point out that sales of fluoride toothpaste in the United Kingdom were less than 5% of total sales in 1970, but rose to more than 95% of sales in 1977. They quote unpublished annual data from unfluoridated parts of Gloucestershire, collected from 1964 onwards, which show substantial improvements in children's teeth before the use of fluoride toothpaste became significant.

Many of the studies in the Netherlands, reviewed by Kalsbeek¹¹, were carried out to evaluate the effectiveness of the school

dental health programme. Temporal reductions in DMFT of about 50% occurred between 1970 and 1980, whether or not the children had taken part in the dental health education program. Kalsbeek also reviewed the use of fluoride tablets and toothpaste and concluded from the data that "factors other than the effects of different fluoride programmes must play a role."

The study in the partly fluoridated city of Auckland, New Zealand¹², examined the influence of social class (which reflects environmental and lifestyle factors, such as diet) as well as fluoridation on dental health as measured by the levels of dental treatment received by children. The paper showed that treatment levels have continued to decline in both fluoridated and unfluoridated parts of the city and that these reductions are related strongly to social class, there being less caries in the "above average social rank" group than in other children. Thus the main ethical argument for fluoridation, that it should assist the disadvantaged, is not borne out by this study.

Fluoridation's benefits

On 15 December 1980, the Dental Health Education and Research Foundation, one of the main fluoridation promoting bodies in New South Wales (NSW), issued a press release entitled, "Fluoridation dramatically cuts tooth decay in Tamworth"¹³. This document, which highlighted results of a study conducted by the Department of Preventive Dentistry, Sydney University, and the Health Commission of NSW, stated in part:

Tamworth's water supply was fluoridated in 1963, and the last survey in the area was conducted in August 1979. It shows decay reductions ranging from 71% in 15-year-olds to 95% in 6-year-olds. . . . All those surveyed were continuous residents using town water.

The "95%" reduction actually corresponded to a reduction in DMFT from 1.3 in 1963 to 0.1 in 1979¹⁴, which is 92%. The press release implied incorrectly that all this reduction was due to fluoridation. However, it has been claimed ever since

Table 2 Extent of fluoridation in Australia, 1977 and 1983

State or territory	Capital city	Year city fluoridated*	% Of state fluoridated† in 1977	% Of state fluoridated† in 1983
ACT	Canberra	1964	100	100
Tasmania	Hobart	1964	74	77
NSW	Sydney	1968	81	81
WA	Perth	1968	83	83
SA	Adelaide	1971	71	70
Victoria	Melbourne	1977	0.7 then 73	71
Queensland	Brisbane	Not fluoridated	10	5

* Each capital city has the majority of the population of its state or territory.

† That is, the percentage of population of state/territory which drinks fluoridated water. Data from Annual Reports of Director-General of Health, for example ref. 17.

the commencement of fluoridation that the maximum possible benefits from fluoridation are obtained in children who have drunk fluoridated water from birth. Six-year-olds would have done this by 1969, when, according to the published data¹⁵, they had a DMFT index of 0.6. The further reduction in caries in optimally exposed 6-year-olds, observed in years following 1969, cannot be due to fluoridation.

Thus, one can say that at best fluoridation could have approximately halved the DMFT rate in 6-year-olds between 1963 and 1969. (Since there was no control population, one could also say that at worst fluoridation might have had no effect in that period.) But from 1969 to 1979, caries in 6-year-olds was reduced a further 83%, by some other factor(s) than fluoridation.

Figure 1 shows that the unknown factors caused in children of each age from 6 years to 9 years similar large reductions in caries. Unfortunately, there are no published data for Tamworth beyond 1979 or in the years between 1972 and 1979, and so it cannot be confirmed whether the large reductions observed^{14,15} from 1972 to 1979 in children aged 10 to 15 were also due to these unknown factors.

A similar reduction beyond the maximum possible for fluoridation is observed for children of each age from 6 to 9 in the published data from Canberra¹⁶, which cover the period from 1964, the stated year of fluoridation, to 1974. In particular, DMFT rates declined by 50% in 6-year-olds from 1970 to 1974 and by 54% in 7-year-olds from 1971 to 1974. These reductions in optimally exposed children cannot be due to fluoridation. Published post-1974 data are needed to check on further reductions in optimally exposed children aged over 9 years.

From 1977 onwards, data have been systematically collected from the school dental services in each Australian state and territory^{9,17}. Table 2 shows the degree of fluoridation in each of these states/territories in 1977 and 1983 and also the dates of fluoridation of the capital cities of these regions. Each of these cities dominates the population of the state or territory in which it lies. The evidence presented in Fig. 2 and Table 2 suggests that states and territories which had been extensively fluoridated for at least 9 years before 1977 (Tasmania, Western Australia and New South Wales) had qualitatively similar large reductions in caries from 1977 to 1983 as a state which was only extensively fluoridated in 1977 (Victoria) and a state which had a small and declining fraction of fluoridation (Queensland). Although the results of the school dental health survey are recorded by age and state, the data have only been published^{9,17,18} so far for ages 6–13 averaged in each state, or for each age for the whole of Australia. There is evidence that the use of fluoride tooth-

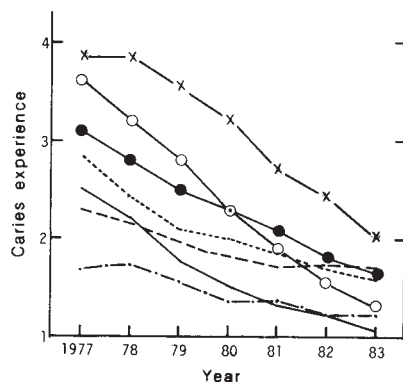


Fig. 2 Decline in the average number of (permanent) teeth per child with caries experience in each Australian state and the Australian Capital Territory as observed in school dental services¹⁷. 'Caries experience' can be one or more decayed, missing or filled teeth, and consists of an average for children aged 6–13 years. See Table 2 for information on the extent of fluoridation in each state/territory in 1977 and 1983 and the year when the main population centre of each state/territory was fluoridated. x, Victoria; O, Tasmania; ●, Queensland; ---, SA; —, NSW; —, WA; —, ACT.

paste in Australia reached a high plateau around 1978, so these observed reductions in caries can be due neither to fluoride toothpaste⁹ nor to fluoridated water.

It is to be hoped that similar data on caries reductions in "optimally exposed" children will be sought in other fluoridated countries. In a region of Gloucestershire, United Kingdom where the main water supply was naturally fluoridated with 0.9 p.p.m. fluoride until 1972, reductions in caries of 51% were observed in 12-year-old children between 1964 and 1979¹⁹. Factors other than fluoridated water must have caused these reductions. After 1972, the main water supply was drawn from a bore with less than 0.2 p.p.m. fluoride, so a recent survey of caries there would be of great interest.

Benefits overestimated?

In some fluoridated areas (for example Tamworth, Australia), temporal reductions in caries have been wrongly credited to fluoridation. The magnitude of these reductions is similar in both fluoridated and unfluoridated areas, and is also generally comparable with that traditionally attributed to fluoridation. Can it be concluded that communities which prefer not to fluoridate, either because of concern about potential health hazards^{20–25} or for ethical reasons (for example compulsory medication; medication with an uncontrolled dose), do not necessarily face higher levels of tooth decay than fluoridated communities? In other words, is it reasonable to ask whether it could be generally true that a major part of the benefits

currently attributed to fluoridation is really due to other causes?

Such a hypothesis would seem to be possible in principle because it is well known that fluoridation is neither 'necessary' nor 'sufficient' (the words between inverted commas being used in the formal logic sense) for sound teeth; that is, some children can have sound teeth without fluoridation, and some children can have very decayed teeth even though they consume fluoridated water²⁵.

To confirm or refute the hypothesis, it is necessary (but not 'sufficient') to examine the absolute values of caries prevalence in fluoridated and unfluoridated areas. If it is true that the absolute values of caries prevalence in some unfluoridated areas are comparable with those in some unfluoridated areas of the same country, then the hypothesis is supported (but not proven), and there would be a strong case for the scientific re-examination of the epidemiological studies which appear to demonstrate large benefits from fluoridation.

The earliest set of studies comparing caries in fluoridated and unfluoridated areas were time-independent surveys of caries prevalence in areas with 'high' natural levels of fluoride in water supplies, conducted by H. T. Dean and others in the United States²⁶. The surveys purported to show that there is an "inverse relationship" between caries and fluoride concentration. From the viewpoint of modern epidemiology, these early studies were rather primitive. They could be criticized for the virtual absence of quantitative, statistical methods, their nonrandom method of selecting data and the high sensitivity of the results to the way in which the study populations were grouped²⁵.

Results running counter to the alleged inverse relationship have been reported from time-independent surveys in naturally fluoridated locations in India²⁷, Sweden²⁸, Japan²⁹, the United States³⁰ and New Zealand^{31,63}. The Japanese survey²⁹ found a minimum in caries prevalence in communities with water F-concentrations in the range 0.3–0.4 p.p.m.; above and below this range, caries prevalence increased rapidly.

These surveys^{27–31} also selected their study regions nonrandomly. But recently Ziegelbecker³² attempted to make a selection close to a random sample by considering 'all' available published data on caries prevalence in naturally fluoridated areas. His large data set, which includes Dean's as a sub-set, comprises 48,000 children aged 12–14 years drawn from 136 community water supplies in seven countries. He found essentially no correlation between caries and log of fluoride concentration. The surveys^{27–32} are generally omitted from lists¹ of studies on the role of fluoridation in caries prevention.

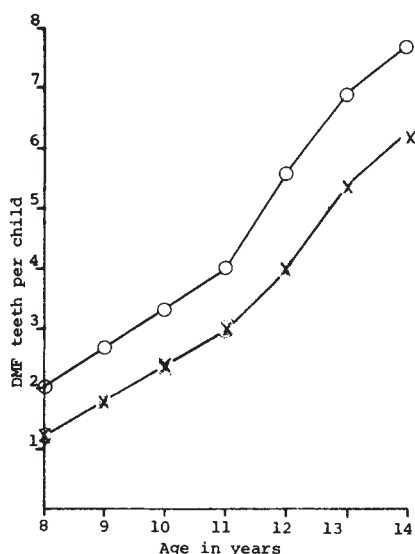


Fig. 3 The variation with age of decayed, missing and filled permanent teeth (DMFT) in fluoridated test towns (x) and unfluoridated control towns (o) in Britain, graphed from data published by the UK Department of Health³³. Note that the rate of increase of DMFT is essentially the same in both groups. Children in the fluoridated areas have an average only one less cavity than children of the same age in the unfluoridated areas.

Further evidence can be drawn from Fig. 2. In 1983, the absolute value of caries prevalence in the Australian state of Queensland (which is only 5% fluoridated) was approximately equal to that in the states of Western Australia (83% fluoridated) and South Australia (70% fluoridated).

The classical British fluoridation trials at Watford and Gwalchmai were longitudinal controlled studies. In this regard they were better designed than the majority of other studies which have been conducted around the world. However, as in the case of almost all other surveys, the examinations were not 'blind'. The review of the British trials by the UK Department of Health after 11 years of fluoridation showed that children in fluoridated towns had approximately one less DMFT (that is, essentially one less cavity) than children of the same age in unfluoridated towns (see Fig. 3). The rate of increase in caries with age was the same in both populations³³.

Thus there are a number of counter-examples to the widely-held belief that "All studies show that communities where water contains about 1 p.p.m. fluoride have about 50% lower caries prevalence than communities where water has much less than 1 p.p.m. fluoride".

At this point the empirical data presented here may be summarized as follows. In the developed world:

- (1) there have been large temporal reductions in caries in unfluoridated areas of at least eight countries;
- (2) there have been large temporal reductions in several fluoridated areas which cannot be attributed to fluoridation;
- (3) the absolute values of caries prevalence in several fluoridated areas are comparable with those in several unfluoridated regions of the same country.

Hence there is a case for scientific re-examination of the experimental design

and statistical analysis of those studies which appear to prove or "demonstrate" that fluoridation causes large reductions in caries. Indeed the few re-examinations which have already been done confirm that there are grounds for concern.

The original justification for fluoridation in the United States, Britain, Canada, Australia, New Zealand and several other English-speaking countries was based almost entirely on the North American studies, which were of two kinds. The limitations of the first set, the time-independent surveys conducted in naturally fluoridated areas of the United States²⁶, have been referred to above.

The second set of North American studies consists of five longitudinal studies—carried out at Newburgh, Grand Rapids, Evanston and Brantford (two studies)—which commenced in the mid-1940s. Only three of them had controls for the full period of the study. These studies were criticized rigorously in a detailed monograph by Sutton³⁴, on the grounds of inadequate experimental design (for example, no 'blind' examinations and inadequate baseline measurement), poor or negligible statistical analysis and, in particular, failure to take account of large variations in caries prevalence observed in the control towns. The second edition of Sutton's monograph contains reprints of replies by authors of three of the North American studies and another author, together with Sutton's comments on these replies. It is difficult to avoid the conclusion that Sutton's critique still stands. Indeed, this was even the view of the pro-fluoridation Tasmanian Royal Commission³⁵. Yet, in major, recent reviews of fluoridation, such as that by the British Royal College of Physicians³⁶, these North American studies are still referred to as providing the foundations for fluoridation, and Sutton's work³⁴ is not cited.

An examination has just been completed of the experimental design of all of the eight published fluoridation studies conducted in Australia. One (Tasmania) is a time-independent survey. Four (Townsville, Perth, Kalgoorlie and the second Sydney study) are longitudinal studies with only two examinations of the test group and either no control or only a single examination of a comparison group. The remaining three studies (Tamworth, Canberra and the first Sydney study) have several examinations of the test group, but no comparison group at all. Thus there has not been a single controlled longitudinal study in Australia. (M.D., to be published). Moreover, it has been shown above that three of the Australian studies (the first Sydney⁴, Tamworth^{14,15} and Canberra¹⁶) inadvertently provide evidence that some other factor(s) than fluoridation is/are playing an important role in the decline of caries prevalence.

Hence the hypothesis that fluoridation has very large benefits requires re-examination by epidemiologists, mathematical statisticians and others outside of the dental profession. The danger of failing to perform scientific research on the mechanisms underlying the large reductions in caries discussed in this paper is that the strong emphasis on fluoridation and fluorides may be distracting attention away from the real major factors. These factors could actually be driving a cyclical variation of caries with time³⁷. It is possible that the condition of children's teeth could return to the poor state observed in the 1950s, even in the presence of a wide battery of F-treatments.

Causes of caries reductions

Many of the authors who reported the reductions in unfluoridated areas acknowledged that the explanation has not yet been determined scientifically^{11,37-41}. It is after all much easier to perform a study which measures temporal changes in the prevalence of a multifactorial disease than to identify the causes of such changes.

Nevertheless, the authors of some of these studies have speculated that important causes of the reductions which they observe might be topical fluorides^{38,53} (such as in toothpastes, rinses and gels), fluoride tablets^{4,38}, school dental health programmes⁹, a lower frequency of sugar intake³⁹, the widespread use of antibiotics which may be suppressing *Streptococcus mutans* bacteria in the mouth⁴¹, the increase in total fluoride intake from the environment^{9,42}, or a cyclical variation in time resulting from as yet unknown causes³⁷.

The present overview has revealed that several of the studies contain evidence against some of these proposed factors. We have seen that the Brisbane study³ and

the Dutch review¹¹ suggest that fluoride tablets may not be important; the Sydney study⁴, one of the British studies¹⁰ and the Dutch review¹¹ each provides evidence against fluoride toothpaste; and the Dutch review¹¹ found no benefit in their school dental health education programmes.

Although there is evidence that fluoride toothpaste cannot be an important mechanism of caries reduction in some of the studies reported here, it must be stated that, unlike the case of fluoridation, there are also a few well-designed randomised controlled trials which demonstrate substantial reductions in caries from fluoride toothpaste⁴³. Hence, the hypothesis can be made that topical fluorides sometimes improve children's teeth, although they are not necessary. So topical fluorides may comprise one of several factors contributing to the solution of the scientific problem of explaining the reduction in tooth decay.

Leverett⁴² has speculated that the caries reductions in his smaller set of unfluoridated locations may be due to "an increase in fluoride in the food chain, especially from the use of fluoridated water in food processing, increased use of infant formulas with measurable fluoride content, and even unintentional ingestion of fluoride dentifrices." This hypothesis cannot explain the reductions in prefluoridation Sydney⁴, or those in unfluoridated parts of Gloucestershire which started in the late 1960s¹⁰. The ingestion of fluoride toothpastes (and gels) by young children is well documented and could account for an intake of about 0.5 mg F⁻ per day in the very young⁴⁴. But the food processing

pathway is unlikely to be significant in western Europe where there is hardly any fluoridation, and infant formulas which are made up with unfluoridated water will give only small contributions. Thus it appears that Leverett's hypothesis may at best be relevant to a minority of the studies listed in Table 1.

Here, the working hypothesis is presented that fluoridation and other systemic uses of fluoride, such as fluoride tablets, have at best a minor effect in reducing caries; that the main causes of the observed reductions in caries are changes in dietary patterns, possible changes in the immune status of populations and, under some circumstances, the use of topical fluorides. Indeed, a promising explanation is that the apparent benefit from fluorides is derived from their topical action. Then, since fluoridated water has a fluoride ion concentration 10⁻³ times that of fluoride toothpaste, its action in reducing caries is likely to be much weaker.

It is known that immunity plays a role in the development of caries, as it does with other diseases. Research is currently in progress to try to develop a vaccine against caries⁴⁵⁻⁴⁷. None of the data presented in the present paper provides evidence against immunity as a factor.

Dentists often argue against changes in dietary patterns as a major factor, on the grounds that sugar consumption has remained approximately constant in most developed countries over the past few decades. However, this is a simplistic argument. First, crude industry figures on total sales of sugar in developed countries con-

tain no information on the distribution of sugar consumption with age and time of day. The form of sugar ingested—for example in canned food, soft drinks or processed cereals—may also be important. Second, tooth decay is increasing together with increases in sugar and other fermentable carbohydrates in the diet in several developing countries^{48,49}. This was also the case with Australian aborigines, even when their water supplies consisted of bores containing fluoride at close to the "optimal" concentration for the local climate^{50,51}. Third, there is more to diet than sugar. For instance, there is some evidence, even conceded occasionally by pro-fluoride bodies⁵², that certain foods which do not contain fluorides (for example wholegrain cereals, nuts and dairy products) may protect against tooth decay. So the whole question of the relationship between total diet and tooth decay needs much greater input from nutritionists and dietitians.

Perhaps the real mystery of declining tooth decay is why so much effort has gone into poor quality research on fluoridation, instead of on the more fundamental questions of diet and immunity.

The main body of this research was performed while the author was a principal research scientist in the CSIRO Division of Mathematics and Statistics, Canberra. □

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Pathogenesis of lentivirus infections

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Following infection of animals or humans, lentiviruses play a prolonged game of hide and seek with the host's immune system which results in a slowly developing multi-system disease. Emerging knowledge of the disease processes is of some relevance to acquired immune deficiency syndrome (AIDS), which is caused by a virus possessing many of the characteristics of a lentivirus.

LENTIVIRUSES, a subfamily of retroviruses (Table 1), derive their name from the slow time course of the infections they cause in humans and animals¹⁻¹⁰. The persistence and spread of these viruses, despite host defences, and the origins and slow evolution of the diseases they cause, pose fascinating problems in pathogenesis which have assumed a new significance with the addition of the agent of acquired immune deficiency syndrome (AIDS) to the subfamily. This review summarizes the increasingly coherent picture of the pathogenesis of lentivirus infections, and discusses the relevance of these concepts to the behaviour and control of the AIDS virus.

Visna-maedi and central issues

Lentiviruses cause chronic diseases affecting the lungs, joints, nervous, haematopoietic and immune systems of humans and animals (Table 1). The prototypic lentiviruses, visna and maedi, derived their Icelandic names from the prominent symptoms (wasting and shortness of breath) of the neurological and pulmonary diseases they cause in sheep. In fact, it is the same virus² that is responsible for both maedi, the more prevalent pulmonary disease, and visna, a paralytic condition showing some resemblance to multiple sclerosis¹¹⁻¹⁵. These two diseases reached epidemic proportions in Iceland in the 1940s about a decade after the virus was inadvertently introduced into Iceland by sheep imported from Germany. An Icelandic physician, Bjorn Sigurdsson, investigated the outbreaks of visna and maedi, showed that a filterable agent caused them¹², and discovered the long incubation period and protracted course of disease that distinguish slow infections¹⁶. Sigurdsson introduced the term slow infection¹⁷ to capture the novel timescale of disease and the experimental design needed to demonstrate transmissibility, thus setting the stage for the discovery of slow diseases in man¹⁸.

In natural and experimental infections⁷ of sheep (Fig. 1a), the aetiological agent of visna-maedi replicates at the site of entry (the lung in natural infections) and subsequently spreads via the bloodstream or by other routes, such as the cerebrospinal fluid (CSF). The infected animal mounts a defensive response which has both nonspecific components, such as phagocytic cells, and specific humoral and cellular-immune elements^{19,20}. These defence mechanisms are effective against extracellular virus but are generally unable to eradicate the infectious agent altogether. Virus persists in many organ systems and continues to circulate in blood and tissue fluids. In the lungs and central nervous system (CNS), tissue is destroyed in areas where inflammatory cells have collected, and eventually this burden of pathological change becomes apparent as shortness of breath or partial paralysis and weight loss. In natural infections, animals generally become symptomatic in the second year of infection and die after a protracted and progressive illness.

This brief description of the major events of infection in visna-maedi is intended to bring out three salient problems in understanding the pathogenesis of slow infections: (1) How does

virus persist and spread in the face of a vigorous and sustained immune response by the host? (2) What causes destruction of tissue? (3) Why do these pathological events evolve so slowly?

Virus gene expression and persistence

The best current explanation for the persistence of lentiviruses is the immunologically silent nature of the infection. Most infected cells harbour the virus in a latent state in which viral antigens are not produced in sufficient quantities for detection and destruction of the infected cell by immune-surveillance mechanisms²¹. To show the magnitude of these damping effects, evidence for and molecular measures of the restricted virus gene expression *in vivo* have been set against a background of the growth of virus under permissive conditions in tissue culture in Figs 1 and 2 and Table 2. In tissue culture, visna virus reproduces rapidly to high titre and destroys the host cell^{22,23}. In this lytic and productive cycle, genetic information is transferred from the RNA genome of the infecting virus to a DNA intermediate

Table 1 Retrovirus subfamilies

Subfamily	Disease	Natural hosts
Oncoviruses	Cancer	Man, animals, birds and reptiles
Spuma viruses	Inapparent infections	Man, animals
Lentiviruses	Slow infections	Man, animals
Visna-maedi virus	Pneumonia, meningoencephalitis	Sheep, goats
Progressive pneumonia virus (PPV)	Pneumonia	Sheep, goats
Caprine arthritis encephalitis virus (CAEV)	Arthritis, pneumonia, meningoencephalitis	Goats, sheep
Zwoegerziekte	Pneumonia, meningoencephalitis	Sheep
Equine infectious anaemia virus (EIAV)	Fever, anaemia	Horses
AIDS virus (HIV)	Immune deficiency, encephalopathy, myelopathy	Man

The retrovirus subfamilies shown are currently accepted taxonomic divisions¹²¹. The EIAV and AIDS virus are provisionally included in the lentivirus subfamily because they, too, cause slow infections and have other properties in common with visna-maedi¹²²⁻¹³¹: cell fusion and other cytopathic effects in tissue culture; virion morphology; polypeptide composition; large envelope glycoproteins; shared antigenic determinants in the major structural protein (gag); similar size and structure of their genomes; nucleotide and amino-acid sequence homologies largely confined to conserved regions of *gag-pol*.

in the cell^{24,25} which serves as a template for the synthesis of thousands of copies of genomic and messenger RNAs (Table 2a). The latter are translated into millions of copies of structural virion proteins²⁶ and infectious progeny (50–100 per cell) subsequently assemble at the cell surface. The infected cells degenerate, either individually or after fusion²⁷, within 3 days.

By contrast, replication of virus in animals is highly focal and unproductive, even in relatively homogeneous populations of cells which serve as substrates for permissive growth in tissue culture: the number of copies of viral RNA in choroid plexus of infected animals is about two orders of magnitude less than in infected choroid plexus cells in culture (Table 2). Synthesis of viral RNA, antigens and virus is confined to one in a hundred to thousands of cells (Table 2b)²⁸. This focal and restricted growth cycle has been revealed by methods for quantitative analysis of virus replication at the single-cell level (*in situ* hybridization)²⁹ and, more recently, by methods that combine macroscopic screening of tissue with single-cell resolution (Fig. 2)³⁰. The fundamental question of why virus growth should be so different in cells in culture from that in tissues is unanswered, but viral RNA synthesis has been defined as the major point in the virus growth cycle at which virus gene expression is blocked²⁸.

Visna thus has two kinds of life cycle, somewhat analogous to bacteriophage λ in *Escherichia coli*: a productive and lytic life cycle *in vitro* and a latent life cycle in sheep, figuratively referred to as lysogeny on a grand scale³. This is probably overstated, as visna virus is not integrated into the host cell genome, at least in tissue culture³¹, but it does epitomize the notion of the clandestine state of the virus as the mechanism by which it eludes the host's defences.

Trojan horse mechanism

A similar mechanism can be invoked to explain the continued spread of virus in the bloodstream, cerebrospinal fluid and other fluids that contain immune cells and neutralizing antibody. In this mechanism, a mobile cell, predominantly if not exclusively a monocyte^{32,33} in the case of visna virus, conceals the virus genome and conveys it without detection to other sites. Evidence for this Trojan horse mechanism again comes from *in situ* hybridization analyses of CSF, where infected cells are concentrated³². (The frequency of leukocytes carrying visna virus in the bloodstream—about 1 in 10⁶ cells—is too low to be detected directly.) The CSF data³² provide evidence of (1) restricted levels of viral RNA accumulation in monocytes; (2) circulation of the latently infected monocytes in tissue fluids containing neutralizing antibody; and (3) transfer of infectious virus between monocytes and choroid plexus cells under conditions of close contact between the cells. These data satisfy the major predictions of the Trojan horse hypothesis.

Inapparent infections and latency

The theme of restricted virus gene expression is the dominant motif of lentivirus infections. In animals, infections are frequently not apparent, with no obvious pathological sequelae for periods that approximate the normal lifespan of the host. In the United States, for example, lentivirus infection of sheep is widespread and probably largely asymptomatic³⁴. There are flocks of sheep in Germany in which antibodies to virus are present in 50 per cent of the serum samples³⁵ and in which clinical symptoms of maedi have never been observed. Importation of silently infected sheep like these from Germany was presumably responsible for the outbreaks of maedi and visna in Iceland⁴.

The AIDS virus also establishes persistent and non-cytopathic infections in normal human lymphocytes³⁶. In the persistently infected cultures, virus production may be absent or limited (but can be induced), in keeping with the definition of latency³⁷. By extrapolation, similar virus–host cell interactions provide a mechanism for the AIDS virus to escape neutralization and

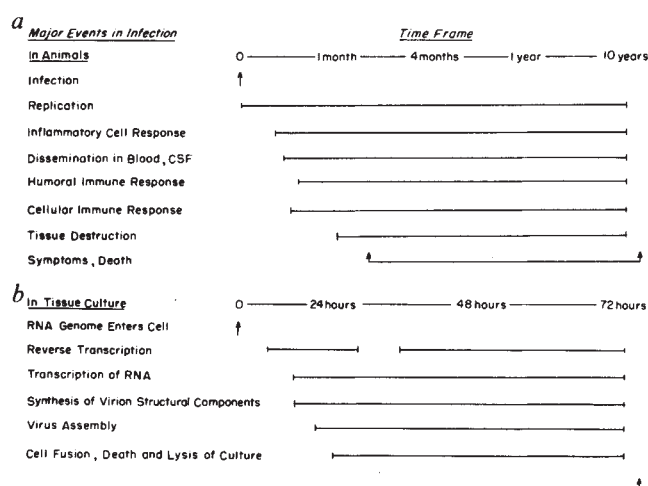


Fig. 1 Time course of animal lentivirus infection. The major events and strikingly different timescale of infection in animals (a) and tissue culture (b) can be seen.

other defences, but also a basis for the comforting observation that inapparent infection and a carrier state are common in infected patients³⁸.

Population spread

Animal lentivirus infections are transmitted between animals, probably by virus inside monocytes and macrophages in secretions (little if any cell-free virus is detectable). The aetiological agents of visna and maedi enter adult sheep by the respiratory route and lambs by a gastrointestinal route in colostrum^{2,4}. The appearance of disease in epidemical form requires special conditions such as those in Iceland, where sheep from many parts of the island are housed together for several days each year². This practice may have been as important in the rapid spread of the disease as the greater susceptibility of the Icelandic than German sheep to lentivirus infection.

AIDS is transmitted similarly. Virus is introduced into the bloodstream through sexual contact, intravenous drug administration with contaminated needles or administration of blood and blood products³⁶. It is not known whether the virus is transferred inside cells, but this is obviously of great importance in predicting the efficacy of conventional vaccine strategies to interdict further spread of AIDS. As in visna, perinatal transmission is also important, although it is not known whether this occurs *in utero* or postnatally.

There is no evidence of germline transmission of animal lentiviruses from mother to offspring, and endogenous lentiviruses are exceptional³⁹, in contrast to the situation with

Table 2 Comparison of the growth of visna virus in animals and in tissue culture

Type of infection	Cells positive for viral RNA (%)	Average no. of copies of viral RNA per infected cell	Cells with viral antigen (%)
a, Tissue culture (choroid plexus cells 3 days after infection)	90	5,000	90
b, Sheep (choroid plexus, alveolar macrophages monocytes, glial cells; 3 days to 3 weeks after infection)	0.1–2	50–150	0.001

Data from refs 26, 28, 32, 56.

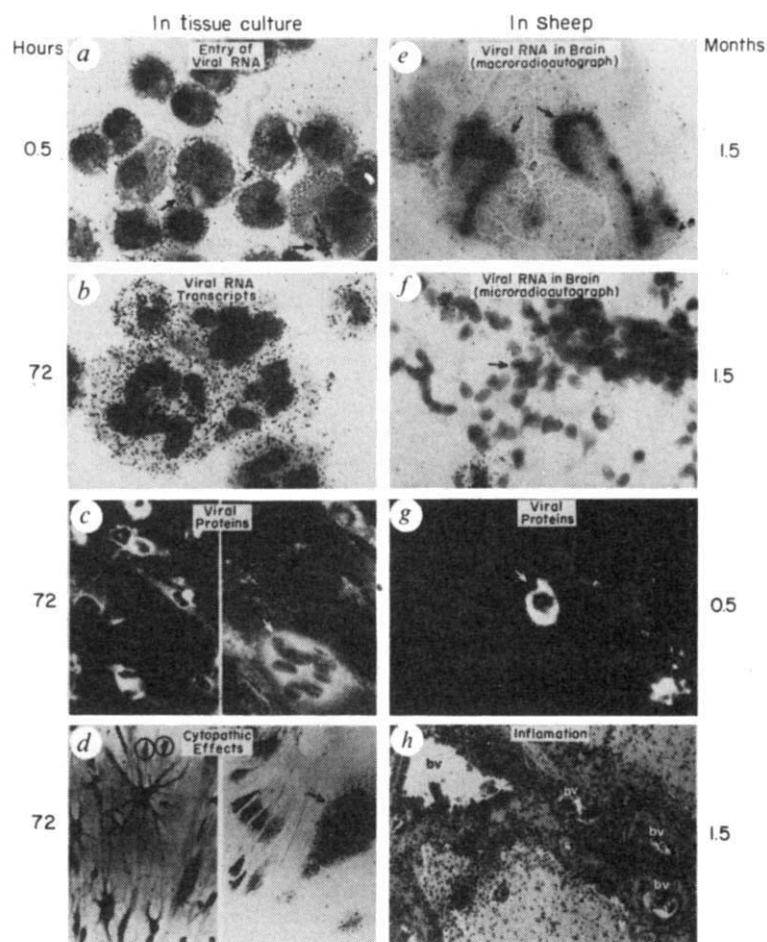


Fig. 2 Life cycles of an animal lentivirus in tissue culture and sheep. *a, b*, Autoradiographs of visna virus RNA detected by *in situ* hybridization. The number of copies of viral RNA in individual cells is proportional to the number of grains over the cell and the length of exposure²⁹. At 0.5 h after infection (at a multiplicity of infection of 3 plaque-forming units per cell) about 30 copies of viral RNA enter the cell, often apparently inside a vacuole (arrows, *a*). By 72 h the cells contain about 5,000 copies of viral RNA²⁶, often in giant cells. Visna virus RNA can be localized anatomically in infected animals to regions surrounding the ventricles (arrows, *e*) using combined macroscopic-microscopic screening³⁰. In this method whole brain sections are hybridized to a virus-specific probe labelled with ¹²⁵I. The γ emission of ¹²⁵I produces a latent image of viral RNA on X-ray film placed against the tissue section after hybridization. The single-cell location of viral genes is determined from a microscopic autoradiograph produced by coating the section with nuclear track emulsion. The Auger electrons emitted by ¹²⁵I produce latent images with good resolution at the cellular level. The average copy number in the cells in the brain is about two orders of magnitude less than that in infected tissue culture (for example, number of grains over the cell in tissue culture 72 h after infection requires an exposure of a few hours; for a probe of the same specific activity, about 3 weeks would be needed to produce the number of grains in the cells in brain)³⁰. In tissue culture there are $\sim 10^6$ copies of major viral protein (gag) easily detected in most cells by immunofluorescence with monospecific antibodies (*c*; the arrow indicates a giant cell with the visna gag polypeptide in the cytoplasm)^{4,127}. Only rare cells with viral antigens can be detected in the infected animal (*g*). The infection in tissue culture leads directly to cell death with lysis of the culture in 72–96 h (*d*). Arrows indicate giant cells in various states of degeneration; individual dying cells are circled. In animals, tissue damage accumulates over months to years (*h*) and is indirectly caused by inflammatory cells (the dark-staining lymphocytes, plasma cells, monocytes, macrophages that surround the blood vessels (bv) and collect in foci in the tissue of this section of brain from a paralysed sheep^{1–7}.

oncogenic retroviruses. The exogenous nature of lentiviruses may reflect their infrequent opportunities to interact with the host genome. Only a few cells are infected in the animal, replication is restricted²⁸ and integration may be rare³¹. The failure of the viral genome to form covalent linkages with the host chromosome probably has a structural basis, as the topological precursor for retrovirus integration is likely to be circular DNA⁴⁰, and in cells infected with visna virus circular molecules are rare (the predominant structure is a nicked or gapped linear duplex)⁴¹. That is not to say that integration of lentivirus genomes cannot occur—integrated as well as unintegrated forms of AIDS virus⁴², equine infectious anaemia virus (EIAV)⁴³ and caprine arthritis and encephalitis virus (CAEV)⁴⁴ have been described.

Variant and common determinants

Antigenic variation is an additional or alternative mechanism for the persistence and spread of the lentiviruses. In this instance, the emergence of mutant viruses with an altered envelope glycoprotein, the antigen to which neutralizing antibody is directed⁴⁵, is the postulated mechanism by which virus temporarily escapes immunological inactivation. Gudnadottir's proposal that 'antigenic drift' might account for the survival of visna virus for such a long time in infected animals² was subsequently supported and fully developed by Narayan and his collaborators. They showed that antigenically distinct viruses could be isolated from sheep persistently infected with visna virus⁴⁶, and that these variants arise by point mutations in the *env* gene, which encodes the virion envelope glycoprotein^{45,47}. In visna,

however, variants do not replace the infecting serotype by antibody selection and, in most long-term infections, the inoculum virus strain persists and spreads without the emergence of antigenic variants^{48,49}. For these reasons it seems unlikely that antigenic variation is a necessary or important means of dissemination of visna virus.

For EIAV, the case for antigenic variation as an important mechanism of virus dissemination is more persuasive. There are cycles of virus replication in which cell-free virus is isolated from serum or plasma, and each new isolate from a cycle is refractory to neutralization by antibody that neutralized previous isolates^{50,51}. This immunologically dictated succession fulfils the expectations of the antigenic variation model for persistence and spread of viruses, and, as in visna virus infections, occurs primarily through point mutations in the *env* gene⁵². But this is not the sole basis for persistence and spread. By the end of the first year, cyclic replication of EIAV is superseded by an inapparent carrier state in which continued dissemination of virus within the host, or to new hosts, is again accomplished by latently infected macrophages⁹. Because this conversion to a Trojan horse mechanism of spread may be a consequence of production of antibody to all strains of EIAV, identification of these common antigenic determinants for neutralization would clearly be important in designing broadly effective vaccines.

It would be premature to predict the role of antigenic variation in AIDS infections, but isolates of the AIDS virus from different individuals differ genotypically⁵³, and this genomic diversity is greatest in the region of the *env* gene⁵⁴. If these genetic changes

are mirrored phenotypically, it will pose serious problems for vaccine development and provide an additional mechanism for the AIDS virus to persist and spread in individuals and populations.

Immunopathogenesis

Pathological changes in lentivirus infections are for the most part indirectly mediated by the immune and inflammatory response of the host. In visna, the coordinate reduction of inflammation and tissue lesions⁵⁵ with immunosuppression suggests that it is the inflammatory cell response (Fig. 2) that causes tissue damage. In the brain, this process is demyelinating¹¹⁻¹⁵ because at least one of the infected cell targets is the oligodendrocyte⁵⁶, the cell in the nervous system which provides the myelin sheath (identified unambiguously by a new method that combines immunocytochemistry, cell-specific antibodies and *in situ* hybridization⁵⁷; Fig. 3). The rare infected cell containing viral antigen probably provokes and sustains this inflammatory response⁵⁶, which then causes tissue damage by mechanisms that are not understood in any detail but could involve interleukin-mediated amplification of the response with indiscriminate damage to uninfected cells ('innocent bystanders') in the area. Lesions in the lungs and joints of infected animals in maedi virus and CAEV infections are also the result of the exuberant inflammatory response. In the lung, infiltration of lymphocytes and monocytes into the alveolar wall interferes with gas exchange; in the joints^{58,59}, the inflammatory cell infiltrate leads to the destruction of the cartilage perhaps by activating chondrocytes to elaborate matrix degrading factors⁶⁰, much as in rheumatoid arthritis.

Both lymphoproliferative changes (enlargement, necrosis) and immune complexes participate in the immunopathology of equine infectious anaemia^{9,61}. The characteristic anaemia is the result of phagocytosis and haemolysis of erythrocytes that first become coated with a viral haemagglutinin and then with anti-virus antibody and complement. Circulating immune complexes of EIAV and antibody also elicit fever and cause glomerulonephritis when they are deposited in the kidney. The, as yet unexplained, glomerulosclerosis and thrombocytopenic purpura of AIDS may similarly be immune complex diseases^{62,63}.

Immunopathology, however, cannot account for all the manifestations of lentivirus infections. There is an inflammatory component in the progressive encephalopathy that occurs frequently in children and adults with AIDS⁶⁴⁻⁶⁶, but in AIDS encephalopathy and myelopathy⁶⁷, inflammation is overshadowed by vacuolation and degenerative changes that are more like those in a paralytic disease of mice caused by some types of murine leukaemia viruses⁶⁸.

Immunodeficiency and cachexia

The agent of AIDS differs most profoundly from the lentiviruses of animals in its effects on the immune system. Immunodeficiency is the hallmark of AIDS^{10,69}, whereas in animal lentivirus infections the immune response is relatively normal or selectively impaired. (In natural US infections, particularly with CAEV, animals ordinarily produce little if any neutralizing antibody⁷⁰.) Until recently, the reasons for this distinction seemed relatively straightforward: the AIDS virus homes to a receptor on the surface of helper T cells^{71,72} and was thought to cause AIDS by destruction and depletion, or dysfunction, of this central element of the immune system. The animal lentiviruses infect monocytes in such small numbers that the impact on immune function is minor³².

It is becoming increasingly evident that this view is oversimplistic, inasmuch as infection of T4 lymphocytes (about 1 positive cell in 20,000-100,000 by *in situ* hybridization⁷³) is as infrequent in AIDS as it is in visna. To account for the large effects on the absolute number of T4 cells and immune function in AIDS, more complex mechanisms need to be invoked, including some that recall the theme of immunopathology in lentivirus

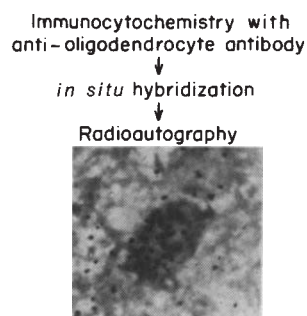


Fig. 3 Determination of virus host cell tropism by the simultaneous detection assay⁵⁷. Brain sections from a paralysed sheep infected with visna virus were reacted with anti-oligodendrocyte antibody, and the reaction was visualized by immunocytochemical methods. After *in situ* hybridization with a visna virus-specific probe, the developed autoradiograph was examined to locate cells (oligodendrocytes) which stained with the antibody and also had an increased number of silver grains such as that shown in the figure. This method identifies the oligodendrocyte unambiguously as one of the types of cells infected by visna virus⁵⁶.

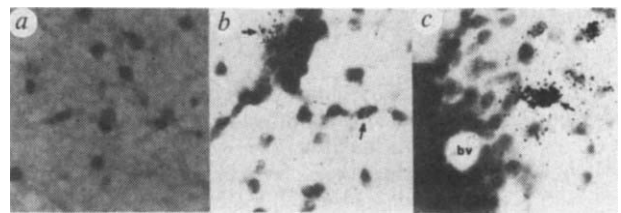
infections. For example, viral envelope glycoprotein shed from productive cells could make large numbers of uninfected cells targets for an autoimmune response directed to viral antigen bound to the T4 receptor. Alternatively, the network of interactions in the immune system should in time lead to an anti-idiotypic response to antibody to the viral glycoprotein. This anti-idiotypic antibody might also be directed against the T4 antigen and viral receptor. This hypothetical reconstruction of the immune response provides a rationale for understanding the production of anti-lymphocyte antibodies in AIDS⁷⁴, and a basis for a paradoxical and potentially hazardous approach to treatment by immunosuppression⁷⁵. The latter could also theoretically relieve a block in the expansion, differentiation or function of T4 cells mediated by another subset of lymphocytes infected with the AIDS virus. Just as plausible, however, are direct mechanisms leading to immunodeficiency, for example, continued recruitment of uninfected T4 lymphocytes into the infectious process, destruction of precursors or the release of immunosuppressive virion components analogous to the p15E protein of feline sarcoma virus^{76,77}.

One of the unexplained symptoms of infection, the cachexia or wasting ('slim disease' is the name given to one form of AIDS in Uganda⁷⁸), might be partly attributable to interactions of lentiviruses with macrophages. In addition to inanition from concurrent enteropathic infections (possibly including AIDS virus) and diarrhoea which contribute to these manifestations of the infection, lentiviruses might, as the result of infection of monocytes, cause release of cachexin, a factor that inhibits adipocyte gene expression and the production of lipogenic enzymes⁷⁹.

Form and tempo of pathology

Visna virus gene expression is markedly curtailed in most infected cells in tissues²⁸, but there are a few cells in which levels of viral RNA approach those in productively infected tissue culture⁵⁶. These gradations in gene expression (Fig. 4) are tightly correlated with detection of viral antigens, the intensity of the inflammatory response, and tissue damage, as would be expected if gene expression determines the form and tempo of pathology. At one end of a spectrum of virus gene expression are a silent majority of infected cells with minimal levels of viral RNA and antigens, enabling the virus to persist and spread. Towards the other end of the spectrum is the occasional cell with higher levels of viral RNA and the antigens responsible for the continuing inflammatory cell response and inadvertent destruction of tissue at a rate commensurate with the scanty production of the inciting antigens. At the extreme end of the spectrum is the lytic

Fig. 4 Quantitative *in situ* hybridization used to demonstrate correspondence between virus gene expression and inflammatory response. Brain sections from a paralysed sheep infected with visna virus were hybridized with a virus-specific probe. In the developed and stained microautoradiographs there is a gradient in gene expression that correlates with the intensity of inflammation. *a*, A region with no inflammation and background levels of hybridization; *b*, as the number of copies of viral RNA increases, larger numbers of grains appear over cells (arrows) adjacent to small collections of dark-staining mononuclear cells; *c*, the largest numbers of inflammatory cells around blood vessels (bv) and tissue are adjacent to infected cells (arrow) with grain counts equivalent to hundreds to thousands of copies of viral RNA typical of productive infections in tissue culture. Viral antigens are occasionally demonstrable in cells with the highest concentrations of viral RNA⁵⁶.



infection in tissue culture, where destruction of cells is a rapid and direct result of virus growth under permissive conditions of replication. These cytopathic effects are more likely to be due to the abundance of virion components (probably the envelope glycoprotein) capable of fusing and killing the cell from within and outside²⁷ than to the high concentrations of extrachromosomal DNA which have been advanced as one explanation for these cytotoxic effects of retroviruses⁸⁰; experimental conditions can be contrived in visna-infected tissue culture (blocking superinfection with antibody)⁸¹ where fusion and cell death occur at DNA concentrations equivalent to those in tissues²⁸ at which cytopathic effects are never observed.

Regulatory mechanisms and control

The major thrust of this review on lentivirus pathogenesis is that the major issues of persistence, spread and the rate and mechanisms of cell death translate into more objective questions about the factors that control lentivirus replication. Indeed, the central fact and unresolved mystery in visna is why the replication of virus is restricted in the same relatively homogeneous and non-dividing population of cells that supports abundant replication after explantation²¹ or in non-dividing cells in cultures derived from the same tissues⁸¹. Many factors have been investigated that might potentially account for restriction; they fall into two general categories (extracellular and intracellular), and pertain to tissue culture systems or living hosts. The latter is an important distinction, because there are many examples of regulatory mechanisms that operate in tissue culture but bear little relationship to reality. For instance, one can establish persistent infections in tissue culture with visna virus in which the cells contain high concentrations of viral antigens, whereas the concentrations are low in infected sheep (A.T.H., unpublished). Moreover, antigenic variants of visna appear in a logical succession based on antibody selection *in vitro*⁸², but not in infected animals^{48,49}; and gene dosage effects in tissue culture⁸¹ apparently do not operate *in vivo*⁸³. The discussion below therefore concentrates on regulatory mechanisms which meet the test of relevance at the organismal level and which might be exploited in the control of lentivirus infections.

Sanctuaries and vaccines

In natural and experimental visna, dissemination of virus by extracellular routes largely ceases with the appearance of neutralizing antibody¹⁹, acting perhaps in concert with viral inhibitory factors in serum and CSF^{84,85}. Neither humoral nor cellular immunity, however, plays a significant part in maintaining latency, since immunosuppression does not relieve the restriction in virus gene expression^{55,86}.

The experience with animal lentivirus vaccines is unfortunately too limited to give much direction to vaccine development. Veterinary practices of identifying infected sheep by serological testing and removing them from flocks have been successful in controlling lentivirus infections of animals^{1,7,87}. No vaccine for

visna or maedi is available because of intractable difficulties in developing an inactivated virus or envelope component vaccine that will induce neutralizing antibody. Vaccinated sheep do produce complement-fixing antibody but are not protected. Moreover, natural infections are transmitted to lambs by cells in the colostrum of mothers carrying neutralizing antibody. This mechanism of transmission and the possibility of cell-cell spread⁸⁸ would circumvent conventional strategies for disease control. Lentiviruses also take refuge from immune defences in the central nervous system. Thus, vaccines alone cannot be expected to be wholly effective in preventing lentivirus infection, but may favourably alter the outcome by reducing at the outset of infection the number of cells with the potential to contribute to pathological changes.

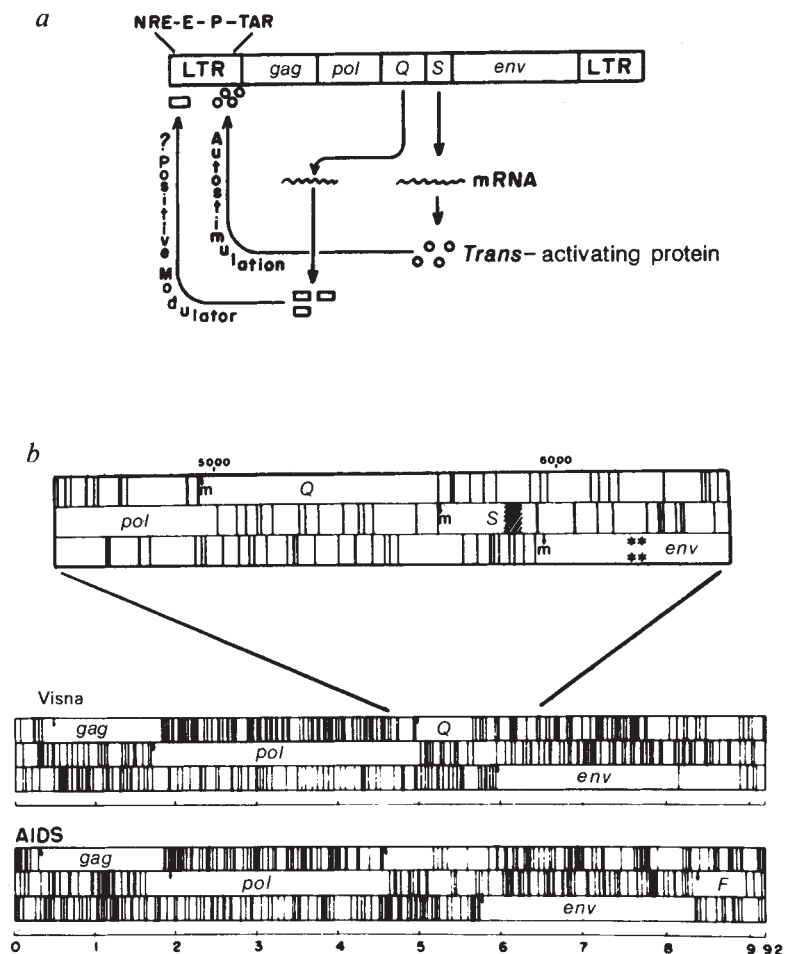
In AIDS, neutralizing antibody^{89,90} titres are low⁹⁰, virus in semen⁹¹ and saliva⁹² is partly cell-associated⁹³, and the brain is infected as a potential sanctuary for virus in a significant segment of the infected population^{64,94-96}. These considerations, and potential difficulties in vaccine development, underscore the importance of the practical measures and behavioural advice currently recommended to limit the spread of AIDS⁹⁶.

Intracellular mechanisms and antisense vaccine

Because viral gene expression directly or indirectly mediates cell damage, measures that limit gene expression should moderate or prevent the pathological consequences of infection. DNA synthesis is a logical point in the virus life cycle at which to intervene with drugs that inhibit reverse transcription or, like interferon, act intracellularly. The experience with the effects of inhibitors of viral DNA synthesis^{97,98} and interferons^{99,100} is limited to *in vitro* experiments which suggest that some benefit might be achieved¹⁰⁰. In AIDS, inhibitors of reverse transcription have already undergone clinical trials, with demonstrable but temporary inhibition of virus replication and little alteration in clinical status¹⁰¹. Sustained treatment with newer and less toxic drugs may induce long-term remissions¹⁰².

Transcription and translation of the lentivirus genome are also potential targets for therapy. The major controlling elements for lentivirus gene expression are located at the 5' end (long terminal repeat, LTR) and centre of the genome (Fig. 5*a, b*). The lentiviral LTR, in common with other retroviruses, has promoter and enhancer domains and two additional domains, designated NRE and TAR in Fig. 5, which have negative or positive effects respectively on gene expression¹⁰³⁻¹⁰⁵. The *trans*-activating region (TAR) is responsive to a gene product encoded in a short open reading frame *S* (Fig. 5*b*)¹⁰³. The *trans*-activating gene product (TAT) of this open reading frame acts post-transcriptionally¹⁰⁶ to stimulate gene expression by 500–1,000-fold and is required for replication^{107,108}. Modest stimulation by *trans*-activation has also been described recently for visna virus¹⁰⁹, which is comparable to that of the AIDS virus when the entire LTR of visna virus is used in the assay (K. Staskus, C. Rosen, W. Haseltine and A.T.H., unpublished). If *trans*-

Fig. 5 Speculations on control of lentivirus gene expression. *a*, Schematic illustration of a prototypic lentivirus genome and a speculative representation of control of gene expression. Transcription of the major genes of the virus is controlled by promoter (P) and enhancer (E) regions in the LTR. A *trans*-activating protein (○) encoded by the short open reading frame *S* binds to a region (TAR) to stimulate gene expression. The gene product of open reading frame *Q* also binds to the LTR to up-regulate transcription. The overall organization of the genomes of visna virus and the AIDS virus is shown in *b* with the controlling region in the centre of the genome with *Q* and *S*. (Modified from refs 103–110.) *m*, Initiator Met codon; *F*, an additional open reading frame in HIV (human immunodeficiency virus); asterisks in *env* indicate the hydrophobic signal sequence.



activation in visna is also effected post-transcriptionally, it could not account for the restricted gene expression in infected sheep which is primarily a result of diminished accumulation of viral RNA²⁸, but it could account for the discrepancy between the relatively high concentration of virus RNA in some cells and the lack of infectivity⁸³.

The reduction in transcription in infected animals was most recently speculated to be due to repressor activity of a putative DNA-binding protein, the basic and hydrophilic gene product of conserved open reading frames (*Q*) in visna¹¹⁰ and the AIDS virus^{111–114}. But there is now evidence that the *Q* gene product is a second positive regulatory factor, because deletion of *Q* slows the growth of the AIDS virus and delays the development of cytopathic effects¹¹⁵. Highly productive and rapidly progressive lytic infections may require co-expression of both factors, as might occur, for example, during activation of macrophages (CAEV) or lymphocytes^{116,117}.

One possible approach to the control of lentiviruses that anticipates the importance of the regulatory proteins encoded by the *Q* and *S* genes, and exploits antisense in mRNA^{118–120}, is the development of defective virus vectors which would stabilize the dormant state of infection. In one such project in progress in the visna model (K. Staskus, E. Retzel and A.T.H., unpublished), the *trans*-activating gene in infectious viral DNA is replaced by its antisense counterpart. Defective virus, produced by co-transfecting cells with the viral antisense DNA and with *S* gene DNA in an inducible expression vector, will have an identical host range to wild-type virus. It will therefore serve as a vector to introduce the antisense gene into cells already harbouring standard virus. In the co-infected cells, *trans*-activation should at first drive production of additional defective virus to

disseminate the antisense vector. Thereafter, the antisense vector should halt *trans*-activation and return the infected cells to a latent state. Of course, before there could be any assurance that the pathological consequences of infection may be prevented, this approach must overcome some theoretical and practical difficulties—particularly superinfection barriers in cells producing AIDS virus and the need to establish co-infection in a large pool of cells with a non-replicating virus.

Conclusions

The lentiviruses are responsible for slow infections of animals and man. Investigations of animal lentivirus infections have previously been directed to the inherently interesting issues of pathogenesis raised by this novel class of infections, parallels to such chronic diseases of man as multiple sclerosis and rheumatoid arthritis, and, to a much lesser extent, practical means of diagnosing, treating and preventing disease. With the knowledge that the causative agent of AIDS is a distant relative of the animal lentiviruses, these objectives have assumed a new and compelling urgency. Restricted gene expression is the general underlying mechanism in the persistence and spread of lentiviruses and the slow evolution of the diseases they produce. This restriction maintains the lentivirus genome in the cell in a covert state, and justifies the optimistic prediction that the commonest form of infection in AIDS, inapparent infection, will prevail for much, if not all, of the natural lifespan of the host. On a more pessimistic note, there is no guarantee that chronic infections will not ultimately progress to some form of illness; or that conventional approaches to prevention and treatment will be successful. These approaches may be frustrated by the ability of all lentiviruses to survive in intracellular and organ

(brain) sanctuaries or to elude host defences through antigenic variation. The prospects of developing vaccines to neutralizing determinants common to all strains of virus, designing vectors to maintain silent infections, and the general progress in lentivirus and AIDS research, are grounds for the hope that these problems will be resolved.

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Pb isotope evidence in the South Atlantic for migrating ridge-hotspot interactions

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Variations of Pb isotopes in basalts erupted along the Mid-Atlantic Ridge suggest that heterogeneities in the upper mantle beneath the South Atlantic are highly structured and dominated by distinct east-west channels connecting the off-ridge plumes with the westward-migrating ridge. These preferential flows are superimposed on a broad radial dispersion of the St Helena and Tristan plumes into the asthenosphere before the ridge overrode these plumes.

WE have recently discovered the presence of short-wavelength geochemical and bathymetric anomalies along the Mid-Atlantic Ridge (MAR) axis just opposite the off-ridge Tristan da Cunha, St Helena and Circe hotspots^{1,2}. We proposed that these anomalies are the result of preferential flow along sublithospheric channels which have developed between the mantle upwelling zones (hotspots) and the westward-migrating MAR since it overrode these hotspots ~80 Myr ago. The ridge in this case acts as a sink and the plume a source of mantle materials. The model was initially developed by Morgan³ for the Galapagos and the Reunion hotspots, purely on the basis of tracks left by anomalous constructional volcanism on the sea floor. The model has received support from the observation that the length of these geochemical anomalies and the magnitude of the associated residual elevation anomalies along migrating mid-ocean ridges both generally decrease as the square root of distance between these migrating ridge segments and related hotspots⁴.

In the South Atlantic, we have also noted that the large geochemical dispersion observed within these spike-like geochemical anomalies may be the result of irregular mixing along these lateral flow channels, between the plume-derived material and the large-ion lithophile element (LILE)-depleted asthenosphere. Furthermore, as the South Atlantic hotspots and the LILE-depleted asthenosphere have distinct signatures in Sr, Nd and Pb isotopic space^{5,6}, a test of the connecting flow channel model and proposed mixing can easily be made by measuring the isotopic composition of basalts derived from these regions. Distinct mixing trajectories are predicted in such isotopic space¹. This is also possible with Pb isotopic ratios alone. In this case, the mixing along the connecting channels between each of these hotspots and the LILE-depleted asthenosphere source should form distinct binary mixing arrays on two-dimensional Pb isotopic ratio diagrams, as the Tristan-Gough-Discovery hotspot(s) have Pb isotopic signatures distinct from that of St Helena and the source of typically LILE-depleted mid-ocean-ridge basalts (MORB)^{5,6} (Fig. 2b). However, no prediction can be made for the Circe mixing trend since this off-ridge hotspot has not yet been sampled. Its influence on the MAR was initially suspected on the basis of geophysical and morphological criteria only¹. Note also that according to these predictions, referred to here as model 1, all binary mixing trends should converge towards the compositional range of the LILE-depleted end-member source for the area (Fig. 2b).

We report on 62 Pb isotope analyses of the same basalts previously analysed for major elements and trace element abundance ratios, such as La/Sm and Nb/Zr, which were instrumental in revealing the spike-like anomalies along the MAR in the South Atlantic (Fig. 1). The Pb isotope analyses were performed on carefully selected fresh glass chips if available; otherwise, pillow interiors were used. All of the samples were carefully hand-picked, cleaned and briefly leached with hydrochloric acid

to remove possible surface contaminants. Our results are listed in Table 1, along with some details of the analytical method. We will refer to the isotopic ratios $^{206}\text{Pb}/^{204}\text{Pb}$, $^{207}\text{Pb}/^{204}\text{Pb}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ as 6/4, 7/4 and 8/4, respectively.

Pb isotope test

For comparison with our model 1 predictions, Fig. 2a shows our actual Pb isotope results plotted on an 8/4 versus 6/4 diagram. As a first-order independent discriminant, we have divided the MAR basalts from 2° S to 47° S into four geographical populations on the basis of the La/Sm variation previously reported¹ (see Fig. 1b). These populations are: 2–12° S, including the Circe spike-like anomaly; the 12–22° S segment, including the St Helena spike-like anomaly; 22–30.75° S, an apparently 'normal' ridge segment with (chondrite-normalized) $(\text{La}/\text{Sm})_{\text{N}} < 0.75$, showing a slight increase southward; and 30.75–47° S, a segment which includes the Tristan-Gough-Discovery spike-like anomaly. The exact boundaries between these four geographical populations were chosen where the $(\text{La}/\text{Sm})_{\text{N}}$ appears to reach a minimum (Fig. 1b). Using these populations, Fig. 2a shows that the St Helena-MAR population (12–22° S) follows a linear array pointing towards the field occupied by the off-ridge St Helena hotspot, and the 30.75–47° S population shows a broad cluster slightly elongated towards the field occupied by the off-ridge Tristan-Gough-Discovery hotspots. However, the 2–12° S MAR population does not form a single linear array; instead at least two linear arrays seem required by the data. The subpopulation from the 7–12° S segment comprising the Circe spike anomaly branches out from that of the St Helena array towards higher 8/4 ratios, presumably pointing towards values representing the Circe off-ridge hotspot rather than the nearby Ascension island. The Pb isotope data reinforce our previous inference^{1,4} that the influential hotspot in this region is located east rather than west of the MAR and may be beneath Circe Seamount¹. The origin of Ascension Island remains uncertain. Rather than being a hotspot itself, its formation may be related to tectonic adjustment in the region, involving MAR rift jumps and migration caused by the Circe hotspot, as well as perhaps the presence of the Ascension fracture zone^{7–9}. In contrast, the subpopulation from 2–7° S (just north of the Circe anomaly) is among the least radiogenic Pb found in ocean-floor basalts, and is indistinguishable from that of the St Helena binary mixing array in the 8/4 versus 6/4 diagram, but, in fact, would show a higher slope on a 7/4 versus 6/4 plot (using data from Table 1). The same grouping and mixing array patterns just described in terms of 8/4 versus 6/4 would also be evident in the 8/4 versus 7/6 diagram, which includes the variation of all four isotopes of lead. On the other hand, there is more scatter and the mixing arrays are not as readily separated in the 7/4 versus 6/4 diagram, because the Tristan and apparently the Circe mantle plume sources lie closer to the

Table 1 Lead isotope ratios* in dredged basalts from the Mid-Atlantic Ridge, 2–47° S

Sample†	Lat. (°S)	Long. (°W)	Depth (m)	²⁰⁶ Pb/ ²⁰⁴ Pb	(±)‡	²⁰⁷ Pb/ ²⁰⁴ Pb	(±)‡	²⁰⁸ Pb/ ²⁰⁴ Pb	(±)‡
EN061 2D-1g	2.24	12.40	3,885	17.829	(13)	15.456	(12)	37.319	(37)
EN061 3D-1	3.43	12.22	3,045	17.804	(23)	15.443	(20)	37.278	(48)
EN061 4D-1g	4.27	12.20	2,300	17.681	(13)	15.422	(11)	37.119	(27)
EN061 5D-1g	5.18	11.52	3,300	17.762	(13)	15.440	(11)	37.239	(27)
EN061 5D-3A	5.18	11.52	3,300	17.640	(9)	15.417	(8)	37.077	(20)
EN061 6D-1g	6.31	11.32	3,218	17.899	(6)	15.462	(6)	37.368	(15)
EN061 7D-1g	7.51	13.46	4,025	18.888	(20)	15.582	(18)	38.517	(45)
EN061 8D-1g	8.01	13.40	3,390	18.925	(32)	15.560	(27)	38.525	(68)
EN061 8D-1	8.01	13.40	3,390	18.972	(11)	15.602	(9)	38.641	(23)
EN061 8D-2A	8.01	13.40	3,390	18.426	(10)	15.520	(9)	37.975	(22)
EN061 9D-1	8.61	13.27	2,205	19.079	(18)	15.595	(16)	38.922	(44)
EN061 10D-1	9.11	13.32	2,663	18.983	(18)	15.597	(15)	38.715	(38)
EN061 10D-2	9.11	13.32	2,663	18.880	(7)	15.575	(7)	38.548	(16)
EN061 10D-3	9.11	13.32	2,663	18.864	(11)	15.599	(9)	38.526	(23)
EN061 11D-1A	9.62	13.23	1,680	19.286	(11)	15.625	(9)	39.097	(24)
EN061 12D-1	9.93	13.12	2,205	19.151	(15)	15.583	(12)	38.875	(30)
EN061 13D-1	10.55	13.01	3,288	18.403	(12)	15.531	(10)	38.095	(40)
EN061 14D-1g	11.05	13.04	3,438	18.245	(13)	15.505	(11)	37.832	(29)
EN061 15D-1	12.03	14.41	3,253	18.605	(14)	15.552	(13)	38.109	(42)
EN061 16D-1g	12.68	14.66	3,575	18.642	(7)	15.535	(6)	38.183	(18)
RC16 6D-1	13.47	14.75	2,752	18.231	(6)	15.489	(5)	37.858	(13)
				18.225	(12)	15.476	(10)	37.814	(26)
RC16 7D-1g	14.08	14.46	3,534	18.207	(12)	15.492	(11)	37.730	(25)
EN061 17D-1g	14.66	13.49	2,862	18.625	(21)	15.558	(18)	38.176	(43)
EN061 18D-1g	15.46	13.26	2,860	18.881	(4)	15.546	(4)	38.403	(12)
EN061 18D-1	15.46	13.26	2,860	18.870	(7)	15.537	(6)	38.372	(16)
EN061 18D-2A	15.46	13.26	2,860	18.364	(17)	15.482	(14)	37.892	(35)
EN061 19D-1g	16.82	14.32	3,837	18.549	(14)	15.546	(12)	37.995	(29)
EN061 20D-1g	17.33	14.18	3,465	18.518	(10)	15.521	(9)	37.971	(22)
EN061 22D-1g	18.38	12.84	3,675	18.727	(7)	15.546	(6)	38.091	(15)
EN061 23D-1g	18.98	12.30	3,537	18.786	(28)	15.563	(24)	38.291	(58)
				18.803	(20)	15.587	(14)	38.371	(22)
EN063 2D-5g	21.50	11.82	3,460	18.316	(13)	15.511	(12)	37.836	(28)
EN063 7D-5g	23.49	13.39	3,630	18.328	(4)	15.500	(3)	37.840	(9)
EN063 9D-5	24.52	13.36	4,010	18.295	(16)	15.511	(13)	37.965	(33)
EN063 10D-5g	24.95	13.19	3,105	18.337	(11)	15.504	(10)	37.941	(23)
EN063 12D-5g	26.00	13.90	2,670	18.307	(17)	15.504	(14)	37.925	(40)
EN063 14D-5g	26.99	13.52	3,610	18.336	(18)	15.520	(11)	37.888	(41)
EN063 14D-5	26.99	13.52	3,610	18.304	(11)	15.504	(11)	37.924	(28)
EN063 17D-5g	28.54	12.54	3,240	18.164	(15)	15.524	(13)	37.941	(33)
EN063 22D-5g	30.50	13.77	3,125	18.254	(14)	15.499	(11)	38.013	(24)
EN063 24D-5g	30.98	13.46	3,530	18.347	(9)	15.511	(8)	37.983	(36)
EN063 23D-5g	31.51	13.41	3,400	18.322	(17)	15.517	(14)	37.983	(35)
AII107-7 25-1	31.83	13.58	2,349	18.272	(13)	15.507	(12)	38.013	(27)
				18.265	(6)	15.496	(6)	37.986	(14)
AII107-7 25-1B	31.83	13.58	2,349	18.290	(8)	15.531	(7)	38.096	(18)
AII107-7 25-2	31.83	13.58	2,349	18.324	(10)	15.548	(9)	38.124	(22)
AII107-7 20-3	33.71	14.25	1,759	18.134	(3)	15.517	(3)	37.948	(9)
AII107-7 18-28	34.55	15.15	2,694	18.122	(3)	15.493	(3)	38.091	(8)
AII107-7 17-71g	35.28	15.73	3,386	18.315	(3)	15.540	(3)	38.268	(10)
AII107-7 15-3g	36.56	17.59	2,459	18.245	(10)	15.539	(9)	38.235	(22)
AII107-7 14-43g	37.19	17.52	2,399	18.258	(7)	15.544	(6)	38.307	(14)
AII107-7 14-77	37.19	17.52	2,399	18.318	(13)	15.544	(10)	38.435	(27)
AII107-7 13-1	37.83	17.14	2,199	18.120	(17)	15.486	(16)	37.961	(33)
AII107-7 10-1g	38.88	16.24	2,169	18.172	(17)	15.535	(14)	38.215	(35)
AII107-7 9-38	39.70	16.05	2,473	18.151	(18)	15.488	(17)	38.108	(34)
AII107-7 7-10g	40.44	16.75	2,612	18.164	(9)	15.500	(8)	37.977	(26)
AII107-7 7-3	40.44	16.75	2,612	18.177	(13)	15.503	(12)	38.001	(30)
AII107-7 6-20g	41.25	16.60	2,394	18.100	(7)	15.539	(6)	38.076	(20)
AII107-7 4-4g	42.91	16.37	2,802	18.064	(23)	15.538	(21)	38.082	(54)
AII107-7 2-38	46.21	13.64	2,699	18,070	(10)	15.530	(10)	38.127	(22)
AII107-7 2-53	46.21	13.64	2,699	18.084	(10)	15.549	(6)	38.176	(20)

* The Pb isotopic ratios were normalized on the basis of replicate measurements of NBS SRM981, using Todt *et al.*'s values (ref. 47). Duplicate analyses are on different pieces from the same sample. The discrimination factor averaged 0.97 ± 0.06 (2 standard errors) % per mass unit. The isotopic ratios were measured at URI on a VG Micromass 30B single collector, double focusing, thermal ionization mass spectrometer. A standard ion exchange procedure was used to separate Pb prior to mass spectrometry (ref. 49).

† g in sample identification stands for glass, otherwise pillow interiors.

‡ Errors in parentheses are 2 standard errors of the last significant figures, taking into account within-run precision and the uncertainty in the discrimination correction. Pb blanks are <0.15 ng on sample sizes up to 0.5 g and are negligible.

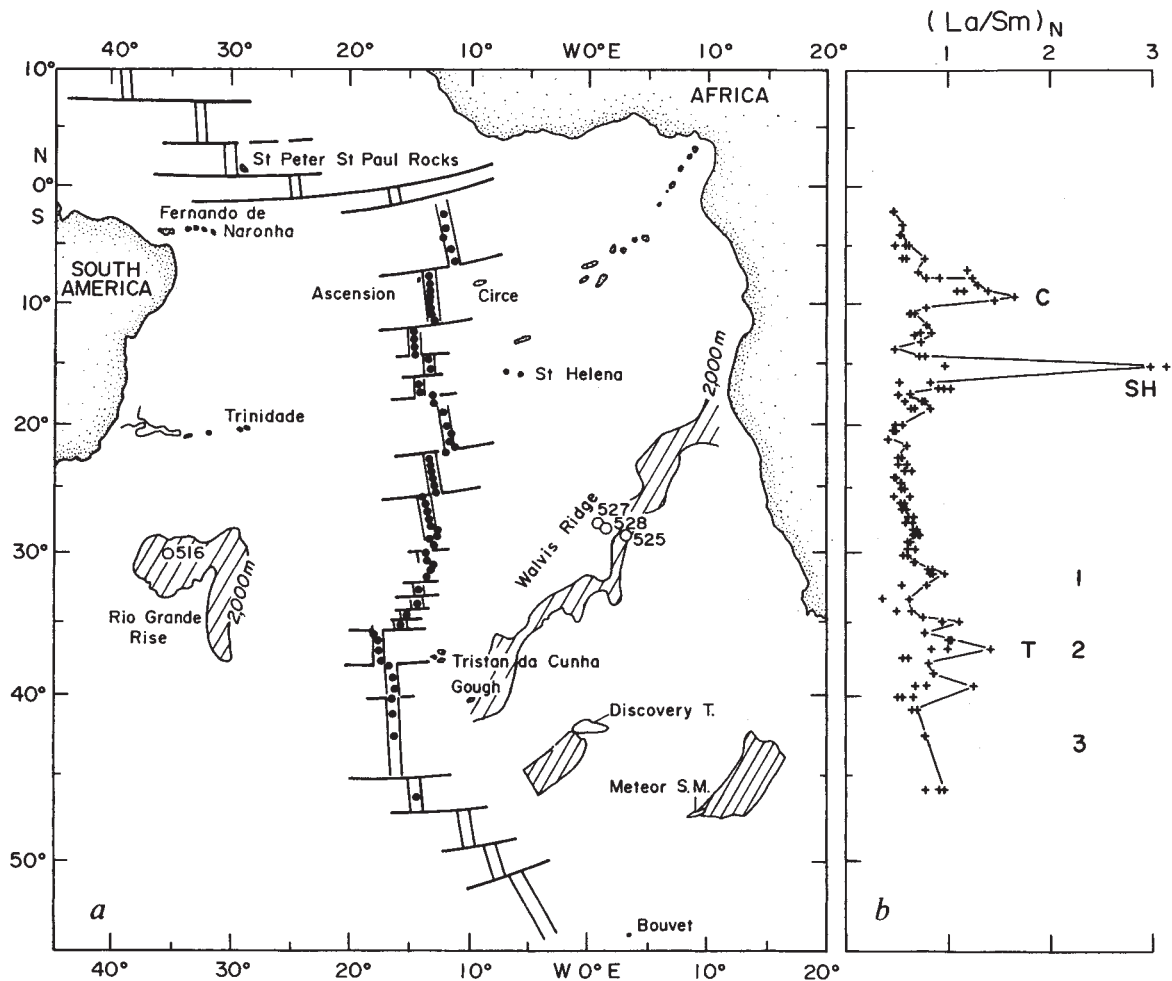


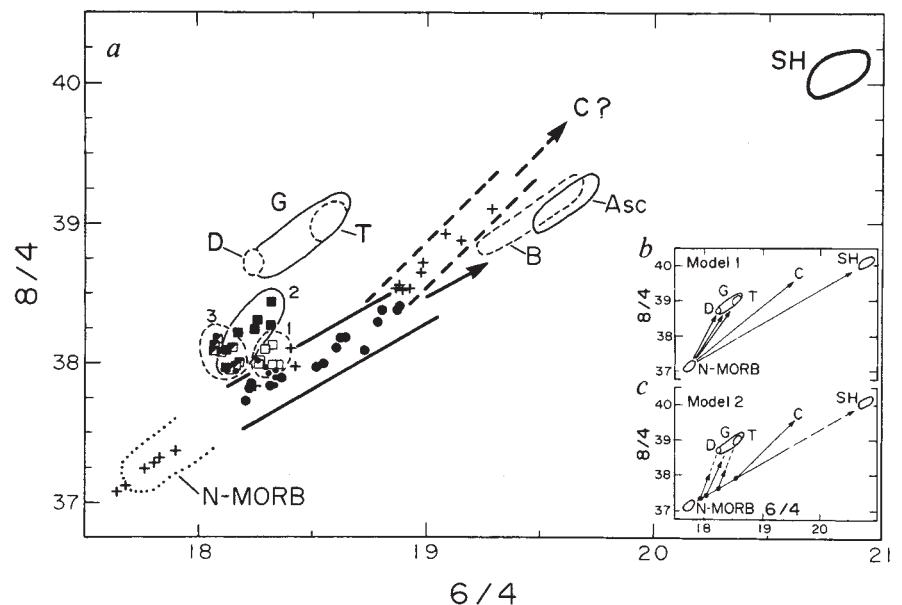
Fig. 1 *a*, Tectonic map of the South Atlantic showing the location of dredged basalts along the MAR, DSDP holes 525A, 528, 527 and 516F (open circles), off-ridge hotspots (islands) and their tracks (hatched areas of seamount chains). *b*, Corresponding latitudinal variation of chondrite-normalized La/Sm in dredged basalts from the MAR-axis. Note spike-like anomalies opposite Circe (C), St Helena (SH) and in the vicinity of the Tristan-Gough-Discovery hotspots (T). Note that there may be at least three distinct small spikes (1, 2, 3) in the latter broader anomaly. These geochemically anomalous ridge segments are also accompanied by anomalous elevation of the ridge axis (see ref. 4); they may reflect lateral sub-crustal plume flow feeding the spreading ridge axis from the corresponding off-ridge hotspots. The analytical uncertainty in the $(La/Sm)_N$ is less than the size of the symbols.

LILE-depleted asthenosphere-St Helena binary mixing array in terms of 7/4. Overall, the mixing systematics described above are self-consistent among the different projections in Pb isotope ratio space. The three distinct arrays are also statistically recognizable in a 6/4-7/4-8/4 three-dimensional space using the method of principal component analysis. This statistical test will be discussed elsewhere (D. A. Fontignie, J.G.S. and B.B.H., in preparation).

Thus, overall, our first-order test of the hotspot source-migrating ridge sink and flow connection is essentially positive in the broad sense that the data indeed reveal distinct binary mixing arrays pointing towards respective fields representing the South Atlantic off-ridge hotspots. However, there are also some important differences. Notably, the three binary mixing arrays do not readily converge towards the field occupied by the LILE-depleted asthenosphere source and thus do not form the fan-tail arrangement of binary arrays initially expected (see model 1 in Fig. 2*b*). Instead, both the Circe binary array comprising the 7-12° S spike population and the 30.75-47° S MAR spike population tend to intersect that of the St Helena-MAR spike population at different levels of 6/4, as if the end-member LILE-depleted asthenospheric source in these regions were already isotopically polluted with a component rich in 6/4, perhaps composed of the St Helena type of material. The details

are more evident when the 30.75-47° S population is subdivided into three segments (30.75-34° S, 34°-39° S, 39°-47° S). This is done on the basis that the migrating MAR could have overridden three different hotspots in the region, Tristan, Gough and Discovery, thus producing three distinct lateral channels; also from the fact that basalts from these three hotspots indeed have distinct 6/4 (Fig. 2*a*); and finally that three small $(La/Sm)_N$ spikes are also observed along the 30.75-47° S MAR segment (spikes 1, 2, 3 in Fig. 1*b*). Figure 2*a* shows that the 30.75-34° S subpopulation forms an array pointing towards the field for Tristan Island, which is geographically located closest to this segment. The southern subpopulation (39-47° S) is subparallel to the former and points towards the field of the off-ridge Discovery Tablemount, which is also closest to this segment. And finally, the MAR segment closest to Gough Island forms another subparallel array intermediate in position between the former two arrays, although overlapping significantly with the other two arrays in terms of 6/4. This is expected as the field occupied by Gough Island basalts reflects a relatively large range in 6/4 which overlaps that of Discovery Tablemount and Tristan Island (Fig. 2). The fact that the north-south zoning along the MAR in terms of 8/4 versus 6/4 space is essentially the same as for the three off-ridge hotspots, indeed, supports the possible presence of a strongly laminated east-west mantle flow pattern,

Fig. 2 8/4 versus 6/4 Pb isotope covariation diagram showing the South Atlantic MAR basalts in relation to: the field occupied by South Atlantic island basalts from off-ridge hotspots, including Discovery (D), Gough (G), Tristan (T), Bouvet (B), Ascension (Asc) and St Helena (SH) (taken from ref. 28); and the field occupied by normal MAR basalts from the North Atlantic (N-MORB) (taken from refs 16, 28, 30, 48). The island fields include analytical uncertainties. Symbols for basalts from the South Atlantic MAR segment subpopulations, based on the distribution of spike-like La/Sm anomalies shown in Fig. 1b, are: 2–12° S, including C-spike (crosses, heavy dashed-line array); 12–22° S, including SH-spike (filled circles, heavy-line array); 22–30.75° S (small dots); 30.75–34° S, including T-1 spike (open squares, thin dashed-line envelope); 34–39° S, including T-2 spike (filled squares, thin full-line envelope); and 39–47° S, including T-3 spike (half-filled squares, thin dashed-line envelope). The analytical uncertainties are less than the size of the symbols. Note: how basalts from the La/Sm spike-bearing MAR-segment subpopulations tend to form distinct linear arrays pointing towards the field occupied by the geographically related off-ridge hotspots; two distinct linear arrays are required by the 2–12° S population. Geographically these correspond to segment 2–7° S (crosses overlapping with N-MORB field), which points towards St Helena or Ascension (but this is not the case on a 7/4 versus 6/4 plot; see also Fig. 5); and the 7–12° S segment (heavy dashed-line array), which points neither towards St Helena nor Ascension Island, but rather towards a field presumably occupied by the large Circe Seamount shown in Fig. 1 (8°15' S, 9°20' W)—presumably the influential off-ridge hotspot at this latitude. Three crosses present between these two arrays (samples EN61 8D-2, 13D-1 and 14D-1g), located on the two lower flanks of the C-spike of Fig. 1b, do not readily fit either of these two arrays (see also Fig. 5). *b*, Idealized binary mixing model 1 initially expected to account for the spike-like geochemical anomalies shown in Fig. 1b. Basalts from each spike would form a vector representing binary mixing between a pure, LILE-depleted, asthenosphere end-member source and plume material of Pb isotope composition characteristic of the off-ridge hotspot sources present at the corresponding latitude of these MAR spikes. *c*, Idealized binary mixing model 2, reflecting a possible interpretation of the Pb isotope distribution actually observed and shown in *a*. Distinct binary-mixing vectors are preserved between the off-ridge plumes and a LILE-depleted end-member which is no longer constant in composition, but is polluted (enriched) to various extents with a St Helena-plume-type end-member material whose contribution increases southward along the MAR. Presumably, the broad background pollution of the LILE-depleted source would have taken place before the migrating MAR sink passed over this hotspot. Black dots at the intersection of the binary mixing vectors D, G and T with the SH-LILE-depleted asthenosphere binary would give the Pb isotope composition of the polluted LILE-depleted asthenosphere at the latitudes of the C, SH and T(-1, -2, -3) geochemical anomalies shown in Fig. 1.



or distinct channels along which binary mixing is predominant, but the LILE-depleted asthenosphere source end-member entering the binary mixture is apparently itself already polluted with a 6/4-rich component (such as St Helena-type material). The influence of this 6/4-rich pollutant apparently decreases southward in some systematic way. An idealized sketch of such an updated mixing model is illustrated in Fig. 2c, where it is referred to as model 2. In this idealization, the pollutant of the LILE-depleted asthenosphere would be of the St Helena type only.

In terms of Pb isotope space, it appears that the MAR basalt variation within the Circe-related spike (2–12° S) and the Tristan–Gough–Discovery-related spike (30.75–47° S) may represent mixtures of at least three pure end-member isotopic components, whereas the St Helena-related spike (12–22° S) is dominantly a mix of only two pure end-member components. Physically, however, the Pb isotope data do not contradict the possibility that along each of the proposed lateral channels and beneath the MAR, the mixing would have taken place between only two types of material, as initially proposed; that is, the surrounding asthenosphere polluted to various extents (depending on the region) and the mantle plume material derived from each of the South Atlantic off-ridge hotspots. An important question which pertains to constraining or deciphering the overall dynamics of the mantle beneath the South Atlantic, is to establish more closely the nature of the background pollutant and map its possible geographical distribution. Is it random? Or does it bear some kind of a relationship with the location of St Helena or some other hotspot? Or, for instance, how does it relate to the so-called DUPAL¹⁰ anomaly? If random, the pollution could, for example, be the result of minor addition of

metasomatic fluids, whereas if it bears some relation to the location of St Helena it could be related to the dispersion of this mantle plume into the surrounding asthenosphere by convection^{11–15}. Insight may also be gained into the mantle flow pattern with depth by comparing our MAR profile with the semi-hemispherical DUPAL anomaly revealed from isotope systematics of ocean island volcanism¹⁰, as it is generally thought that MORBs are sampling a rather shallow upper mantle, whereas ocean island volcanism seems to tap deeper sources.

Asthenosphere pollution

First, we will briefly pursue the consequences of our updated idealized model 2 in Fig. 2c. The intersections of the four binary mixing arrays formed by the MAR anomalies associated with Circe, Tristan, Gough and Discovery with the St Helena–LILE-depleted asthenosphere binary, would provide essentially the mean Pb isotopic value of the polluted asthenosphere end-member present beneath the MAR near the intersection with each of the four proposed connecting channels. Remarkably, Fig. 3 shows that these mean values are inversely related to radial distance from the St Helena hotspot, which would suggest that the extent of pollution of the asthenosphere is related to the St Helena plume location and may be a reflection of its broad dispersion into the upper mantle by convection. Thus, although a significant portion of the plume has been deflected along the connecting lateral channel since the migrating MAR overrode this hotspot, the remaining fraction may at the same time and to a lesser extent have been distributed radially into the upper mantle. The radial dispersion could also have taken place earlier, when the ascending plume was totally intraplate

Fig. 3 Plot of $6/4$ Pb isotopic composition of the hypothetical model 2, LILE-depleted, St Helena-polluted, asthenosphere present beneath the MAR axis at the latitude of the C, SH and T-1, T-2 and T-3 spike-like anomalies shown in Fig. 1 (intersections of the migrating MAR axis with the lateral flow channels from off-ridge plumes located east of the ridge), as a function of radial distance from St Helena hotspot to these intersections. The uncertainties are of the order of the size of the surrounding circles. See Fig. 2c for method used in determining these hypothetical end-member Pb isotopic compositions (dots). The apparent negative correlation suggests that the extent of pollution of the LILE-depleted asthenosphere about the St Helena hotspot, by dispersion, may have been radial in the past (before the migrating MAR axis overrode the St Helena hotspot). Also superimposed is the Pb isotopic variation of basalts with normal ridge segments (that is $(La/Sm)_N < 0.63$ in Fig. 1b). N-MORBs from segments 12.5–14.5° S (large dots); 20.5–30.75° S (small dots), 34.6–40.4° S (squares) tend to follow the negative correlation, but segment 2–7° S is clearly not related by radial dispersion-pollution model 2, suggesting additional complications.

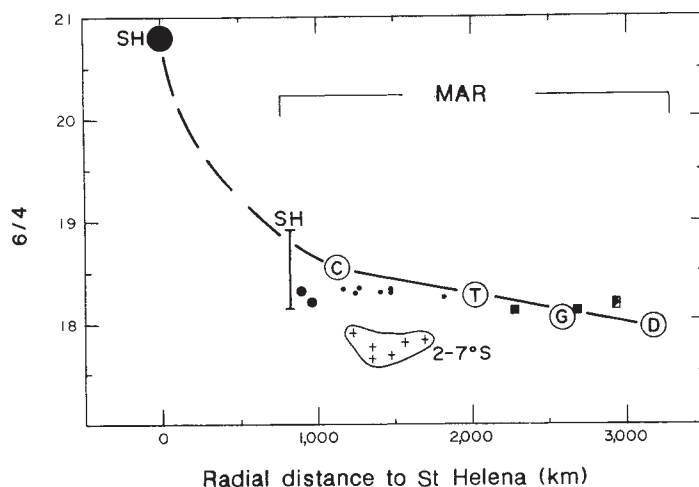
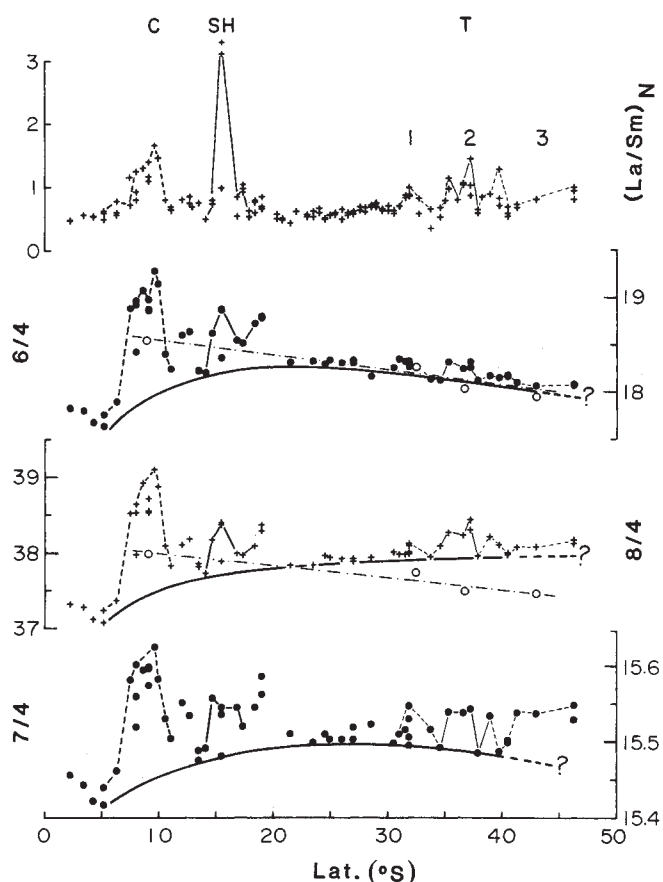


Fig. 4 Comparison of latitudinal variation in $7/4$, $6/4$, $8/4$ Pb and $(La/Sm)_N$ in basalts from the MAR. The analytical uncertainties for $(La/Sm)_N$, $6/4$, and $8/4$ are less than the size of the symbols and less than twice the symbol size for $7/4$. Note that the observed baseline (solid line) for $(La/Sm)_N$ remains essentially constant; that for $6/4$ passes through a maximum around 24° S near St Helena, that for $7/4$ reaches a maximum around 25–30° S, and that for $8/4$ progressively increases southward and levels off over the Tristan region. In contrast, the $6/4$ and $8/4$ baselines consistent with model 2, shown as dash-dot lines through the open circles (from Fig. 2c), decrease linearly southward. The baselines in model 3 are interpreted as being due to background pollution of the LILE-depleted asthenosphere by dispersion in the past of the St Helena and Tristan plumes; presumably before the migrating MAR overrode these hotspots and the lateral off-ridge plume-derived flow channels developed. The observed latitudinal baseline profiles show more complex spectra than predicted by model 2 or by the $(La/Sm)_N$ profile. Note that the St Helena $(La/Sm)_N$ spike-like anomaly is greater than that of Circe but the converse is true for the Pb isotope ratios. This could be taken to suggest an inverse relationship between these parameters when, in fact, and in general, $(La/Sm)_N$ positively correlates with $6/4$, $7/4$ and $8/4$. This artefact is the result of an unusual enrichment of $(La/Sm)_N$ in one of the basalt samples from station 18D (18D-1). The basalt sample is also the most alkalic of our entire South Atlantic MAR basalt population and its $(La/Sm)_N$ probably reflects a smaller degree of melting than usual.



and spread on encountering the rigid lithosphere above. Alternatively, the radial dispersion or pollution could reflect a redistribution due to secondary cellular convection in the upper mantle in the region^{11–15}.

We can further test the radial distribution pattern of the pollutant suggested by our model 2 interpretation in two ways. First, we superimpose in Fig. 3 the Pb isotope variations actually observed along the ridge segments located in between those that contain the $(La/Sm)_N$ spike-like anomalies; that is: the segments from 2–7° S, 12.5–14.5° S, 20.5–30.75° S and 34–35° S. On the basis of the $(La/Sm)_N$ variations, these segments would appear to be essentially 'normal' and unaffected by plumes (that is $(La/Sm)_N < 0.63$ and normal ridge elevation). The Pb isotopic variation along these segments with respect to radial distance from St Helena should follow the same pattern as predicted by our new model 2 interpretation. Figure 3 shows that the match

is generally poor, particularly for the 2–7° S and 12.5–13.5° S segments, suggesting additional complications or some inconsistencies in interpretation. The second test is made by comparing, on one hand, the $(La/Sm)_N$ and Pb isotopic profiles along the MAR and, on the other, the $6/4$ and $8/4$ Pb isotopic baselines observed with those suggested by contamination model 2 (Fig. 4). Between the Circe, St Helena and Tristan-Gough-Discovery spike-like anomalies, the $(La/Sm)_N$ ratio returns to a baseline value that would be considered as typical of normal ridge segments in the North Atlantic or Pacific. In contrast, the baselines for the three Pb isotope ratios do not stay constant. The baseline for $6/4$ goes through a maximum around 24° S, and that of $7/4$ perhaps around 25–30° S, whereas the baseline for $8/4$ keeps increasing southward and tends to level off over the Tristan Platform transect. In contrast to these observations, the model 2 interpretation indicates that the $6/4$ and $8/4$

baselines should decrease linearly southward. The latitudinal Pb isotope ratio profiles thus show more complex spectra than predicted by model 2 or by the $(\text{La}/\text{Sm})_N$ profile, with spikes at the latitude of the hotspots as for the $(\text{La}/\text{Sm})_N$ (resulting from the channelling of the plumes), superimposed on a broader background variation related to dispersion into and pollution of the asthenosphere, perhaps by two rather than a single hotspot. We will refer to the two-background-pollutant model as model 3. Two major background pollutants of the asthenosphere are apparent from the following reasoning. First, the component rich in 6/4 reaches a maximum near 24° S close to St Helena Island, which suggests that its source may indeed be St Helena. On the other hand, the background pollutant component rich in 7/4 and 8/4, which seems to reach a maximum further south in the vicinity of the Tristan-Gough-Discovery hotspots, may be related to this group of hotspots, since these hotspots are also characterized by high 7/4 and 8/4 but relatively low 6/4 (Fig. 2). Such an 8/4- and 7/4-rich pollutant of the asthenosphere has also been observed in the Indian Ocean and has been attributed to pollution by a hotspot with Kerguelen-type Pb (refs 16–20).

The details of the two-component pollution of the asthenosphere source in the South Atlantic (model 3) is best revealed by studying the relative Pb isotopic variation (6/4, 7/4, 8/4) in a triangular diagram, using first only basalts with $(\text{La}/\text{Sm})_N < 0.63$. These MAR basalts lie outside the three noted $(\text{La}/\text{Sm})_N$ spikes and usually would be considered as derived from the so-called LILE-depleted asthenosphere. The location of the transition zone where the St Helena type of pollutant fades out and the Tristan type becomes more dominant, is readily detectable in such a triangular diagram²¹ (Fig. 5a). From 2° S to 24° S the mixing line is essentially binary between the LILE-depleted source and the St Helena type of pollutant, the latter increasing fairly systematically southward to reach a maximum at 24° S, where the St Helena-type pollutant is felt most (see sequence along this trend). South of 24° S, the trend becomes ternary, with the influence of the Tristan-type pollutant becoming progressively more important and the St Helena type decreasing southward, while the ²⁰⁷Pb-rich component characterizing the N-MORB's source stays constant. Further south the trend systematically reverses, as the pollutants from both Tristan and St Helena decrease southward and the contribution from the N-MORB's source increases slightly. Note that the 24–47° S population overlaps the field found for the so-called normal MORBs from the three Mid-Indian-Ocean Ridges^{16–20}. Finally, we note that had the Tristan-type pollutant south of 24° S along the MAR not been present, the trend would have shown a reversal of the sequence along the same St Helena–asthenosphere mixing trend. The systematics observed with respect to the St Helena and Tristan hotspot locations is thus remarkably clear on such a triangular diagram (Fig. 5).

In comparison, MAR basalts located within the anomalous spikes depart from this sequence and form mixing-vector-like trends, which again tend to be directed towards the fields occupied by their respective plume end-members (Fig. 5b). The exact proportions of the two types of plume pollutants in the asthenosphere beneath the MAR sampling profile, or the mixing proportion within the spikes, cannot be more quantitatively described, as this would require knowing the Pb concentration in such plumes which are unknown and which need not be the same. There is also no guarantee that the Pb isotopic composition of the sources of the island basalts are representative of the pure end-member plumes. In fact, the vector-like trend formed by Tristan-Gough Islands and Discovery Tablemount themselves (Fig. 5b) suggests both a progressive southward increase in the LILE-depleted asthenospheric component (relatively rich in ²⁰⁷Pb) and a progressive decrease in the ²⁰⁶Pb-rich St Helena-type pollutant with increasing distance from St Helena Island. Thus, the extent of pollution of the asthenosphere by the St Helena-derived pollutant appears to be spatially widespread

beneath the South Atlantic. It may have partly diluted neighbouring plumes as well, and the pollution may have taken place for quite some time. In fact, the spatial southward decrease in 6/4 pollution is also observable in the past, across the Walvis Ridge, from basalts recovered from the north-south DSDP drill hole sequence 527, 528, 525 (ref. 22) (Figs 2a and 5b). According to the fixed hotspot reference frame²³, these basalts were also erupted beneath the Tristan hotspot and slightly northward.

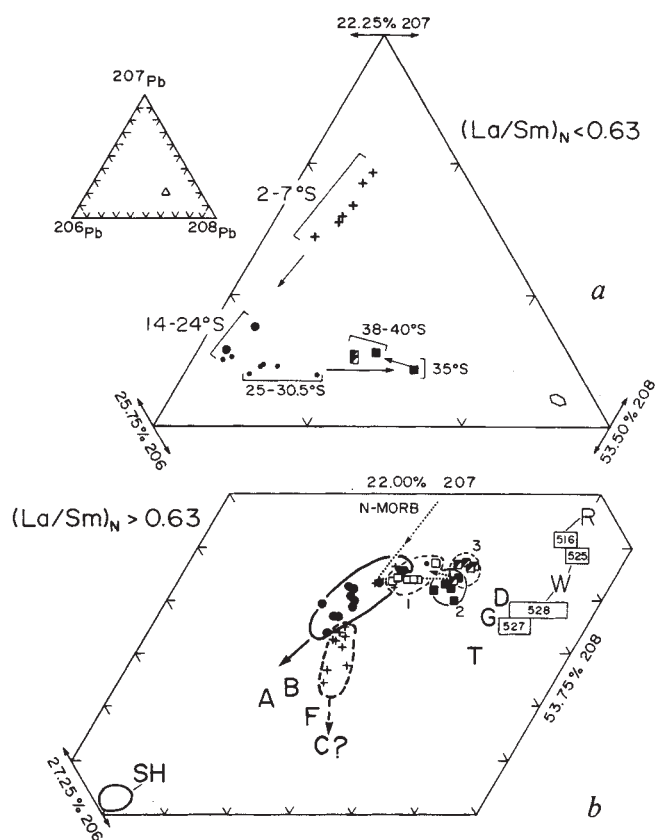
Finally, there is also a suggestion of a temporal increase of the St Helena pollutant with time from the vector-like sequence exhibited in Fig. 5b by lavas from the Rio Grande Rise, Walvis Ridge, Discovery Tablemount, Gough and Tristan da Cunha. For example, basalts from the Rio Grande Rise (produced at the Tristan Hotspot, 86 Myr ago; ref. 24), followed by Walvis Ridge basalts (69–71 Myr; ref. 25), are progressively less polluted in 6/4 relative to modern basalts from Tristan Island ($<10^5$ yr; ref. 26). In this time versus extent of 6/4 pollution sequence, the Discovery basalt (25 Myr old; ref. 27) comes third, and Gough, fourth (2–6 Myr; ref. 26), although in this case the radial distance from these two hotspots to St Helena is greater than from Tristan. A more detailed scrutiny of temporal and spatial relationships is not warranted here, as some of these basalts need first to be age-corrected for radiogenic Pb buildup since erupted (once parent/daughter concentrations become available).

Mantle dynamics beneath the South Atlantic

The concept of pollution of the LILE-depleted asthenosphere by the random dispersion of blobs by convection as they ascend through the asthenosphere, was first suggested by Allègre and co-workers^{15,17–19}. Their arguments are based on a rather coarse sampling of the mid-ocean ridge system in the Atlantic, East Pacific and Indian Oceans at ~100–500 km intervals. Their comparative study shows that the pollutant in the Indian Ocean appears generally to be rich in 8/4 (Kerguelen or Tristan type) relative to the North Atlantic or East Pacific where such a pollutant has not been observed, but instead a pollutant rich in 6/4, similar to St Helena type, may be present and responsible for the elongation of the MORB field in the 7/4 versus 6/4 diagram^{28–32}. These observations have led Allègre and co-workers to suggest the blob cluster model, or provinciality of blob types^{15,17–19}. In contrast, our more detailed sampling (30–100 km intervals) of the North Atlantic³³, the Galapagos^{34–36} and now the South Atlantic^{1,2}, has led us to pursue the more deterministic, fixed plume source-migrating ridge model^{1,3,37}. The Pb isotope evidence presented for the South Atlantic lends some support to both models and helps merge the two models towards describing a more realistic picture of upper-mantle dynamics. Our results show that heterogeneities in the upper mantle beneath the South Atlantic are not randomly distributed, but highly structured and dominated by east-west laminations, with streaks of identifiable isotopic signature corresponding to that of the hotspots located approximately at these latitudes east of the MAR. Thus, the probable existence of lateral sub-lithospheric channels, or tongues, connecting these off-ridge plumes and the westward migrating MAR at shallow depth in the upper mantle is substantiated by our Pb isotope test. An important fraction of the present plume flow around these hotspots is apparently strongly skewed along these distinct channels, which may have developed since the migrating MAR ridge sink has overridden the plumes. Mixing of plume-derived material with the asthenosphere along these channels appears irregular and apparently not purely binary as initially thought on the basis of trace element abundance ratios only, with the possible exception of the MAR–St Helena channel. The depleted asthenosphere beneath the South Atlantic appears to have also been broadly polluted by at least two distinct components, one of the St Helena type, the other of the Tristan type. The degree of pollution is apparently directly related to distance to these hotspots. The St Helena-related pollutant seems to be more

Fig. 5 Triangular plot of relative abundances of Pb 206, 207 and 208 in MAR basalts from the South Atlantic. *a*, With $(\text{La}/\text{Sm})_N < 0.63$ (only N-MORB). Note that N-MORBs from the 2–24° S ridge segment suggest an array which reflects dominantly binary mixing between the LILE-depleted asthenosphere and the St Helena-type plume source (rich in ^{206}Pb), with St Helena's contribution to the mix increasing southward (a pollution model of the asthenosphere dominated by St Helena). In contrast, N-MORBs from segment 25–35° S reflect ternary mixing between the LILE-depleted asthenosphere, St Helena and Tristan (rich in ^{208}Pb) plume-type sources of pollutants, with Tristan's contribution to the mix increasing southward to the detriment of St Helena's, whereas the LILE-depleted source's contribution stays essentially constant. Southward, past Tristan, N-MORBs from segment 38–40° S are still reflecting ternary mixing, but with both St Helena and Tristan's types of pollutants of the LILE-depleted asthenosphere now decreasing southward. The analytical uncertainties are given by the error polygon in the lower right corner of the plot. *b*, With $(\text{La}/\text{Sm})_N > 0.63$ (basalts from the C, SH, and T-1, T-2, T-3 spike highs shown in Fig. 1*b*). Also plotted are averages of basalts from relevant South Atlantic off-ridge hotspots, and range for basalts from DSDP holes 525A, 527 and 528 on the Walvis Ridge (W) and 516F on the Rio-Grande Rise (R) (data uncorrected for age); and for reference, the N-MORB trend of Fig. 5*a* (dotted line). Note how the SH spike points towards the St Helena island field, the T-1, 2, 3 spikes towards Tristan-Gough-Discovery off-ridge hotspot fields, and the C-spike towards values corresponding neither to Fernando De Noronha (F), Bouvet (B), nor Ascension (A), but presumably Circe seamount (C). Note also that the three odd basalts discussed in Fig. 2 are part of the ternary mixing for N-MORBs discussed in *a*, and that the off-ridge Tristan (T), Gough (G) and Discovery (D) hotspots are progressively less enriched in the St Helena, ^{206}Pb -rich, pollutant, moving radially southward from St Helena Island. See text for discussion of DSDP holes.

Symbols used are the same as defined in Fig. 2.



widespread and to have polluted the plumes ascending, apparently at a rate increasing with time, towards the south within the past 80 Myr.

These observations are not unexpected within the framework of the migrating ridge sink-plume source model, which includes three critical stages of plume dispersion into the asthenosphere: (1) intraplate, when dispersion was at a maximum; (2) ridge-centred, when dispersion was at a minimum; and (3) off-ridge-channel-connected, when dispersion is intermediate^{1,4}. According to plate reconstruction models^{23,38,39}, the time-spans for these three stages for St Helena are respectively 75–90, 0–15 and 110 Myr, whereas for Tristan these are 60, 20–40 and 60–80 Myr. Thus, for comparable plume size or flux, the radial dispersion of the St Helena plume is expected to be more widespread than for the Tristan plume.

Within the frameworks of both the blob cluster model¹⁵ and the DUPAL anomaly¹⁰, one could conclude that the MAR region between 24 and 35° S marks the transitional boundary between the 8/4 Pb-rich, Kerguelen-Tristan blob cluster which dominates the Indian Ocean, and the 6/4 Pb-rich St Helena blob cluster which dominates the Atlantic, north of this boundary. However, in detail the pattern of pollution and dispersion is more complicated, apparently of smaller scale and tied to local hotspots. Sampling of the mid-ocean ridge system in the vicinity of the Bouvet triple junction suggests that the influence of the Bouvet plume, whose Pb isotope signature is intermediate between the Tristan-Kerguelen and the St Helena type (Figs 2*a* and 5*b*), can be detected and seems to extend irregularly over ~300–1,700 km along the three plate boundaries branching from the triple junction^{40–42}. Further north, we have seen that the extent of asthenosphere pollution, and the geochemical nature of the pollutants along the mid-ocean ridge system, are closely tied to the types of plume present in their vicinity. This tends

to rule out a background pollution of the asthenosphere beneath the region by random addition of LILE-rich metasomatic fluids migrating from deeper zones of the Earth's mantle. Instead, the background pollution observed is more likely to reflect the dispersion at shallow depths of the South Atlantic plumes. However, the mechanism of dispersion of these plumes (or vertical chains of blobs) remains uncertain. It could be the result of a complex mantle convection pattern in the asthenosphere (see refs 11–15). Or, the dispersion could simply be the result of a radial flow of the plumes discharging into the asthenosphere, particularly when they were totally intraplate and had not yet sensed the effect of the spreading ridge axis (the sink). Or for that matter, both mechanisms may well have been operative⁴³. Our isotopic data does not allow such distinction.

Finally, we consider the observation that the Pb isotopic ratios seem to be more sensitive than the La/Sm or Nb/Zr ratios at detecting the broad background plume-related pollutant along the so-called normal ridge segments, as illustrated by the discrepancies in the baselines shown in Fig. 4. A proper understanding of such discrepancy, which at first may appear paradoxical, is important as it pertains to constraining not only the trace element and isotopic ratios, but also the elemental concentrations in the various plumes relative to that of the LILE-depleted asthenosphere, particularly of the elements in the denominator of these trace element or isotope ratios. In turn, this should help in constraining, or even mapping, the relative mass distribution of the different pollutants affecting the asthenosphere, and help in understanding the mechanisms of dispersion of heterogeneities and mantle dynamics in general. Two factors may contribute to the different sensitivities in detecting asthenosphere pollutants. One stems from mixing, the other from partial melting. First, it should be recalled that in mixing two reservoirs with distinct trace element or isotope ratios, the ratio in the

mixture is usually not linearly related to the mass-fraction entering the mix, unless the concentration ratio 'n' between the plume source (pollutant) and the asthenosphere source, of the chemical species in the denominator of such trace element or isotope ratios is unity^{44,45}. The parameter 'n' controls the curvature of the hyperbolic mixing curves. The greater is 'n', the more readily can a small fraction of the pollutant entering the mixture be detected. Since Pb isotopic ratios are more sensitive in revealing the plume pollutants in the South Atlantic than the (La/Sm)_N ratio (or the Nb/Zr analogue¹), it suggests that 'n' for Pb is greater than for Sm or Zr. In other words, Pb appears to be more incompatible than Sm or Zr. This is apparently also the case around Iceland²⁹, where 'n' for basalts derived from the Iceland plume relative to those derived from the LILE-depleted source south of 60° N is ~4 for Pb but only 1.5 for Sm (ref. 46). Although the ratio 'n' measured in the basalts is not necessarily identical to the ratio in the sources of these basalts, as it is affected by operative degrees of partial melting beneath Iceland and south of 60° N, the relative variation of 'n' among various trace elements is helpful.

Another mechanism suggested by G. Waggoner (personal communication) may be invoked if the plume-derived dispersed heterogeneities (pollutant) affecting the LILE-depleted source beneath the MAR have suffered some melt extraction in the recent past, but before the final partial melting stage taking place directly beneath the MAR axis. If this were the case, incompatible elements which partition preferentially into such melts would also be preferentially removed, thus generating residual plume material (plumes being dispersed towards the ridge) with lower ratios of highly incompatible to moderately incompatible elements (such as La/Sm or Nb/Zr). These residual plumes would thus have intermediate trace element ratios between that of the original plume and the LILE-depleted asthenosphere. In contrast, these dispersed residual plumes would still have a Pb isotope composition similar to that of the original plume even after a 100–200 Myr residence time in the asthenosphere, because of the long half-lives of the radioactive parents U and Th. The more different the trace element or

isotope ratio of the plume-derived pollutant is from that of the LILE-depleted asthenosphere, the more readily can it be detected in the mixture sampled along the MAR, even if the ratio 'n' discussed earlier is unity. The plausibility of this mechanism for the South Atlantic will depend on knowing better the dynamics and thermal structure of the upper mantle, to determine over what depth range the dispersion of these plumes and partial melting may take place. The question also requires a detailed analysis of relative rates, including the ridge migration and horizontal velocities of the plume domains being dispersed both along the proposed channels and radially.

Although the origin of mantle heterogeneities and mechanism of dispersion in the upper mantle remains an open question, we believe that some of the major discrepancies remaining between the different models discussed here may be more a reflection of the different scales of sampling existing along the mid-ocean ridges, rather than being of real substance. The evidence for upper mantle flow lamination in the South Atlantic would probably not have been detected had a sampling at intervals of 100–500 km been used, as currently available in the Indian Ocean^{16–20}. Morgan³ initially developed the model of plume source-migrating ridge sink and connecting channel on the basis of the track of constructional volcanism on the sea floor between the Reunion hotspot and the eastward-migrating Central Indian mid-ocean ridge. It would be of great interest to find out if a denser sampling of the region might reveal, in addition to the general background Kerguelen–Tristan pollutant already observed^{16–20}, some well-defined, spike-like geochemical anomalies along this ridge opposite the Reunion hotspot, as well as opposite other hotspots in the Indian Ocean, as our model predicts and sparse existing data already suggest^{18,19}.

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Identification of a putative second T-cell receptor

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Framework monoclonal antibodies have identified a population of human lymphocytes that express the T3 glycoprotein but not the T-cell receptor (TCR) α - and β -subunits. Chemical crosslinking experiments reveal that these lymphocytes express novel T3-associated polypeptides, one of which appears to be the product of the $T\gamma$ gene. The other polypeptide may represent a fourth TCR subunit, designated $T\delta$.

UNDERSTANDING T-cell recognition of antigen and the restriction of this process by major histocompatibility complex(MHC)-encoded antigens has been an important goal in immunology. A major step forward occurred with the immunochemical identification of clone-specific disulphide-linked heterodimers on T cells, composed of subunits termed TCR α and β (relative molecular mass (M_r) 50,000 (50K) and 40K, respectively)¹⁻³. Genes that rearrange during thymic ontogeny and encode the TCR β ^{4,5} and α ⁶⁻⁸ subunits were isolated either by subtractive hybridization or by probing with oligonucleotides.

A unique feature of the human TCR $\alpha\beta$ was the observed co-modulation², co-immunoprecipitation^{9,10} and required co-expression¹¹ of the TCR $\alpha\beta$ molecules with the T3 glycoprotein, which suggested that these two structures were related. Subsequently, the direct physical association of the two protein complexes was demonstrated by chemically crosslinking the TCR $\alpha\beta$ molecules to the T3 glycoprotein and identifying the components of the crosslinked complex as the TCR β -subunit and the T3 glycoprotein 28K subunit¹². A T3 counterpart is similarly associated with murine TCR $\alpha\beta$ ^{13,14}.

A third gene that rearranges in T cells, designated $T\gamma$, has been identified in mouse¹⁵⁻¹⁷ and man^{18,19}. $T\gamma$ gene rearrangements occur in lymphocytes with suppressor-cytotoxic as well as helper phenotypes and may produce a large number of $T\gamma$ chains¹⁸⁻²³. However, the function of the $T\gamma$ gene is unknown. Furthermore, neither the protein encoded by the $T\gamma$ gene nor its possible association with other structures (as occurs with TCR $\alpha\beta$ and T3 glycoproteins) has been defined.

It appears increasingly likely that the TCR $\alpha\beta$ molecule alone determines both antigen recognition and MHC restriction on at least some T cells^{24,25}. However, it is unclear whether this receptor accounts for the process of T-cell selection during thymic ontogeny or for all antigen-specific recognition by mature T cells. For example, suppressor T lymphocytes remain an enigma; in some cases they delete or fail to rearrange TCR β genes^{26,27}. Thus, it is of great importance to determine whether a second T-cell receptor exists, to define its structure (particularly with regard to the possible use of the $T\gamma$ gene product) and ultimately to understand its function(s).

Here, framework monoclonal antibodies that react with shared determinants on human TCR $\alpha\beta$ molecules were used to identify peripheral blood lymphocytes (PBL) that were T3⁺ but did not react with these monoclonal antibodies. We reasoned that such lymphocytes might express non- α , non- β T3-associ-

ated structures and that such molecules might represent a second T-cell receptor complex. This strategy resulted in the identification of a T3-associated molecular complex on the surface of human lymphocytes that do not express detectable TCR α and β transcripts or polypeptides. We suggest that this complex is a putative second T-cell receptor.

Monoclonal antibodies

A murine framework antiserum that recognizes most human TCR $\alpha\beta$ molecules has been reported previously²⁸. Subsequently, a murine monoclonal antibody, called β Framework 1 (β F1), that is reactive with shared determinants on the human TCR β -chain was obtained (M.B.B. *et al.*, in preparation). The β F1 antibody reacts with most T3⁺ human PBL and is capable of immunoprecipitating the TCR $\alpha\beta$ heterodimer from all human T-cell lines examined that have $\alpha\beta$ T-cell receptors and express the T3 glycoprotein. Immunoprecipitations from a panel of T-cell lines using β F1 demonstrate this reactivity as well as the heterogeneity of the TCR α - and β -subunits from different receptors (Fig. 1a). Like the framework antiserum²⁸, this monoclonal antibody does not stain the surface of living T cells, but will react specifically with both membrane and cytoplasmic T-cell receptors after partial solubilization of the lymphocyte plasma membrane with 70% ethanol. Double staining of human PBL with fluorescein-conjugated anti-T3 monoclonal antibody and biotinyl- β F1 monoclonal antibody, followed by phycoerythrin-conjugated avidin reveals that β F1 recognizes 95-97% of peripheral blood T3⁺ lymphocytes. However, it clearly defines a small population of T lymphocytes that is β F1⁻, yet T3⁺ (Fig. 1c).

A second framework monoclonal antibody, WT31, initially thought to recognize the T3 antigen²⁹, has recently been shown to react with a common epitope of human TCR $\alpha\beta$ ³⁰. While double staining with anti-T3 monoclonal antibody (OKT3) and WT31 revealed that each antibody blocks binding of the other, one-colour fluorescence indicated that WT31 typically recognizes 1-3% fewer cells in peripheral blood than does anti-T3 (data not shown). WT31 efficiently binds to the surface of T cells (for example, in fluorescence-activated cell sorter (FACS) analyses) and is capable of immunoprecipitating the TCR $\alpha\beta$ molecules, albeit inefficiently, from radiolabelled detergent lysates³⁰ (see also Fig. 1b, lane 3). Thus, β F1 and WT31 appear to recognize all but a small fraction of human peripheral blood T3⁺ cells, and define a subpopulation that is T3⁺ but unreactive

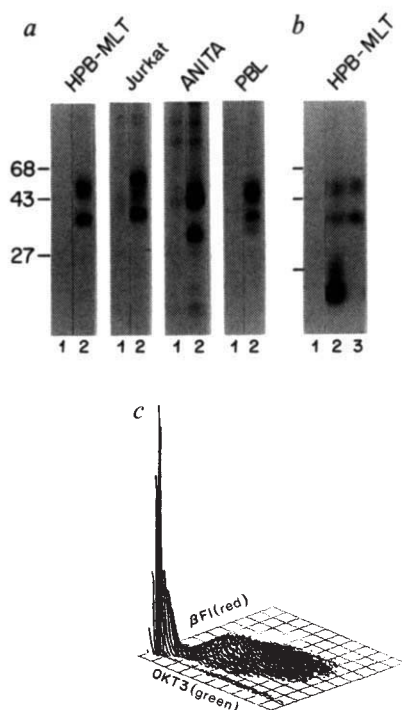


Fig. 1 Reactivity of framework monoclonal antibodies recognizing TCR $\alpha\beta$. *a*, Immunoprecipitates from ^{125}I -labelled lymphocyte lysates were analysed by SDS-PAGE. The radioiodinated T-cell leukaemia lines HPB-MLT and Jurkat, the human T-lymphotropic virus type 1-transformed cell line ANITA and resting PBL were solubilized in 1% Triton X-100 and immunoprecipitated with a control antibody, normal mouse serum (NMS) (lane 1 for each cell line) or framework antibody to TCR $\alpha\beta$, antibody βF1 (lane 2 for each cell line). *b*, ^{125}I -labelled lymphocytes were solubilized in 0.1% Triton X-100 and immunoprecipitated with NMS (lane 1), anti-T3 antibody, UCHT-1 (ref. 40) (lane 2) or framework antibody to TCR $\alpha\beta$, WT31 (lane 3). The efficiency of immunoprecipitation with WT31 was improved at the lower concentration of Triton X-100 used here. *c*, Two-colour FACS analysis of normal adult PBL using anti-TCR $\alpha\beta$ and anti-T3 monoclonal antibodies. PBL were stained first with FITC-conjugated anti-T3 antibody (OKT3) and then with biotinyl-anti-TCR $\alpha\beta$ antibody (βF1) followed by phycoerythrin-conjugated avidin (PE-avidin, Becton Dickinson). Red and green fluorescence were measured in comparison with nonspecific control FITC- and biotin-conjugated monoclonal antibodies (data not shown). Cells unreactive with either antibody are non-T cells (lower left corner); cells that are doubly positive, reacting with both OKT3 and βF1 , make up the large population of lymphocytes in the centre of the grid; cells that are βF1^- but OKT3 $^+$ comprise the narrow group of lymphocytes (4% of the T3 $^+$ cells) observed along the x-axis.

Methods. Viable lymphocytes were isolated by Ficoll-Hypaque density gradient centrifugation for both SDS-PAGE and FACS analyses. For SDS-PAGE, lymphocytes were radioiodinated by the lactoperoxidase technique, solubilized in 1% Triton X-100 and immunoprecipitated using 1 μg of specific antibody (βF1 or UCHT-1) or 1 μl of NMS. Monoclonal antibody 187-1 (3 μg) was used as a second antibody for βF1 and UCHT-1 (ref. 47). The immunoprecipitates were then analysed by SDS (10.5%)-PAGE under reducing conditions. The ^{125}I -labelled molecules were visualized by autoradiography as described previously²⁸. Two-colour cytofluorographic analysis was performed by first staining PBL with FITC-OKT3 for 45 min at 4°C. After washing, the lymphocytes were fixed in 1% paraformaldehyde for 15 min at 23°C and then incubated in 70% ethanol in phosphate-buffered saline (PBS) for 5 min at -20°C. After further washing, the cells were stained with biotinyl- βF1 followed by PE-avidin. Analysis was performed on an Ortho cytofluorograph.

Table 1 Phenotypes of cell lines derived from PBL of immunodeficiency patients

Cell line	Source	Description	% Positive for:			
			WT31	T3	T4	T8
1	IDP1	Allo	50	100	11	50
2	IDP1	WT31 $^+$ sort	100	100	70	28
3	IDP1	WT31 $^-$ sort	0	100	0	62
4	IDP2	Fresh PBL	61	63	38	16
5	IDP2	PHA	100	96	18	80
6	IDP2	Allo	2	100	0	43
7	IDP2	PHA	12	93	1	18

The description of each cell line indicates either the conditions for activation of the lymphocytes, or their source. Alloantigen (allo)-activated cultures were stimulated with irradiated allogeneic PBL at weekly intervals. Mitogen (phytohaemagglutinin, PHA)-activated lines were stimulated with a 1:1,000 dilution of PHA (Difco) at culture initiation. WT31 $^+$ and WT31 $^-$ sorted cell lines 2 and 3 (sort) were obtained from IDP1 cell line 1 by cell sorting analysis. All cell lines were propagated *in vitro* in media composed of RPMI 1640, 10% human serum and conditioned media containing 2–5 units of IL-2 as described previously³⁴. Viable lymphocytes were isolated by Ficoll-Hypaque density gradient centrifugation and stained with 0.5 μg of WT31, OKT3, OKT4 or OKT8 (Ortho, Raritan, New Jersey) for 30 min at 4°C. After washing, the cell pellets were stained again with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse F(ab')₂ fragments. FACS analyses were performed on an Ortho cytofluorograph or a Coulter Epics as described previously³⁷. Specifically stained positive cells were determined relative to a negative control profile for each cell line (stained with nonspecific control monoclonal P3X63Ag8). Cells having fluorescence intensity channel numbers greater than the intercept of the negative control profile with the baseline were counted as positive, and the per cent positive was calculated relative to the total number of cells counted.

with both of these framework monoclonal antibodies against the TCR $\alpha\beta$ molecules. Evidence that the T3 $^+$ lymphocytes that are unreactive with βF1 are also unreactive with WT31 is presented below. We have used WT31 primarily for FACS analyses and βF1 primarily for immunoprecipitation studies.

Isolation of WT31 $^-$ T3 $^+$ lymphocytes

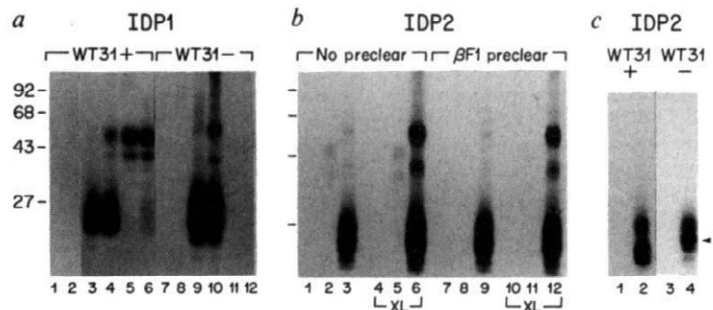
Efforts at growing the WT31 $^-$ T3 $^+$ population from normal adult PBL proved difficult, as the WT31 $^+$ T3 $^+$ lymphocytes usually overgrew the WT31 $^-$ T3 $^+$ cells after mitogenic stimulation. However, growth of the WT31 $^-$ T3 $^+$ population from the PBL of immunodeficiency patients was successful. Immunodeficiency patient 1 (IDP1) suffered from the bare lymphocyte syndrome, and lacked class II MHC antigen expression on lymphoid cells^{31,32}, while immunodeficiency patient 2 (IDP2) suffered from an ectodermal dysplasia syndrome³³ and displayed poor *in vitro* T-cell proliferative responses to mitogens.

After activation of PBL from IDP1 with alloantigen and propagation in conditioned media containing interleukin-2 (IL-2)³⁴, the resultant cell line was observed to be ~50% WT31 $^+$ T3 $^+$ and 50% WT31 $^-$ T3 $^+$ (Table 1, cell line 1). Subsequent sorting of this cell line yielded homogeneous populations of WT31 $^+$ T3 $^+$ cells and WT31 $^-$ T3 $^+$ cells (Table 1, cell lines 2 and 3, respectively).

Cell lines were also obtained from IDP2; 63% of the PBL from IDP2 were T3 $^+$ and 1–3% fewer cells (61%) were WT31 $^+$, which is typical of normal PBL (Table 1, cell line 4). Activation of these IDP2 PBL with either phytohaemagglutinin (PHA) or alloantigen and propagation *in vitro* with conditioned media resulted in several cell lines, including a homogeneous WT31 $^+$ T3 $^+$ cell line (Table 1, cell line 5), a homogeneous WT31 $^-$ T3 $^+$ cell line (cell line 6) and, on a third occasion, a cell line that was 88% WT31 $^-$ T3 $^+$ (with 12% contaminating WT31 $^+$ T3 $^+$ cells) (cell line 7). Note that the WT31 $^-$ T3 $^+$ popula-

Fig. 2 SDS-PAGE of cell surface T3 and T3-associated (crosslinked) molecules from IDP1 and IDP2 cell lines. **a**, IDP1 cell lines 2 (WT31⁺) and 3 (WT31⁻) were ¹²⁵I-labelled as described in Fig. 1 legend. Radioiodinated, intact lymphocytes were then either crosslinked by incubation in PBS (pH 8) containing 50 µg ml⁻¹ DSP (even-numbered lanes) or mock-incubated (odd-numbered lanes). The cells were then solubilized in 1% Triton X-100 and immunoprecipitated as described previously¹². Lanes 1, 2, 7 and 8 were immunoprecipitated with NMS. Lanes 3, 4, 9 and 10 were anti-T3 immunoprecipitates using UCHT-1. Lanes 5, 6, 11 and 12 were anti-TCR αβ immunoprecipitates using βF1. Note that T3-associated molecules (40–55K) on the anti-T3 immunoprecipitates were detected at higher levels in the crosslinked samples than in the non-crosslinked samples. **b**, IDP2 cell line 7 (88% WT31⁺T3⁺) was ¹²⁵I-labelled and either treated with DSP [crosslinked samples (XL) lanes 4–6 and 10–12] or mock-incubated (non-crosslinked samples, lanes 1–3 and 7–9). Immunoprecipitations were performed using NMS (lanes 1, 4, 7 and 10), anti-T3 antibody UCHT-1 (lanes 3, 6, 9 and 12) and anti-TCR αβ antibody βF1 (lanes 2, 5, 8 and 11) either without (left-hand side) or with (right-hand side) preclearing of TCR αβ molecules using βF1. Note that a small fraction of radiolabelled TCR αβ was detected in lanes 2 and 5 (without preclear) but not with the βF1 preclear (lanes 8 and 11). **c**, IDP2 cell lines 5 (WT31⁺T3⁺) and 7 (88% WT31⁺T3⁺) were ¹²⁵I-labelled, solubilized in 1% Triton X-100 and immunoprecipitated using NMS (lanes 1 and 3) or anti-T3 antibody UCHT-1 (lanes 2 and 4). The T3 heavy subunit (27K) appeared similar in these two cell lines, while the T3 light subunits (19–25K) did not (compare lanes 2 and 4 below the arrowhead).

Methods. ¹²⁵I-labelling, solubilization in 1% Triton X-100, immunoprecipitation and visualization after SDS (10.5%)-PAGE by autoradiography were performed as described previously²⁸. Chemical crosslinking of intact radiolabelled lymphocytes using DSP (50 µg ml⁻¹) in PBS (pH 8) was for 30 min at 23 °C, as described previously¹². After immunoprecipitation, all samples were examined by SDS-PAGE under reducing conditions using 5% 2-mercaptoethanol, which cleaved both the disulphide bonds between protein subunits and the DSP chemical crosslink.



tion contained both T4⁺T8⁺ and T4⁺T8⁻ cells (cell lines 3, 6 and 7). Further phenotypic analyses revealed that this population was T11⁺ but negative for natural killer cell markers such as Leu 7, Leu 11 and OKM1 and for the immature thymocyte marker T6 (data not shown).

WT31⁻T3⁺ cells are negative for TCR αβ

Having identified WT31⁺T3⁺ and WT31⁻T3⁺ populations, we sought to determine whether these lymphocytes expressed TCR αβ molecules that could be immunoprecipitated by βF1. While βF1 immunochemically defined a heterodimeric structure on the surface of ¹²⁵I-labelled WT31⁺T3⁺ IDP1 lymphocytes (Fig. 2a, lane 5), it failed to recognize a similar protein on the WT31⁻T3⁺ population from the same individual (Fig. 2a, lane 11). Similar analysis of IDP2 cell lines revealed a trace of TCR αβ on the 88% WT31⁺T3⁺ cell line 7 (Fig. 2b, lane 2) consistent with the 12% contamination with WT31⁺T3⁺ cells. Thus, the WT31⁻T3⁺ cells, identified by the lack of cell surface reactivity with antibody WT31 on FACS analysis, were also βF1⁻, as determined by lack of TCR αβ on immunoprecipitation. Note that all WT31⁺T3⁺ and WT31⁻T3⁺ cell lines expressed similar amounts of T3 as determined by FACS analysis (data not shown) and by immunoprecipitation with anti-T3 antibody (Fig. 2a, lanes 3 and 9, and c, lanes 2 and 4). However, the T3 molecule on WT31⁻βF1⁺T3⁺ lymphocytes was not identical to that on WT31⁺βF1⁺T3⁺ cells, as revealed SDS-polyacrylamide gel electrophoresis (SDS-PAGE). One- (Fig. 2c) and two-dimensional (data not shown) gel analysis indicated that the difference in T3 was restricted to the light T3 subunits, which reproducibly exhibited different SDS-PAGE mobilities (Fig. 2c, arrowhead).

To determine whether the WT31⁻βF1⁺T3⁺ population lacked TCR αβ molecules, or alternatively expressed TCR αβ molecules that failed to react with the monoclonal antibodies used, we analysed the cells for messenger RNAs encoding the TCR α and β proteins. ³²P-labelled complementary DNA clones encoding TCR α, TCR β and Tγ were used to probe Northern blots containing whole-cell RNA from WT31⁻βF1⁺T3⁺ and WT31⁺βF1⁺T3⁺ IDP2 cell lines and from HPB-MLT, which is known to contain mRNAs for TCR α, TCR β and Tγ. No TCR α or β mRNA transcripts were detected in the RNA from the WT31⁻βF1⁺T3⁺ IDP2 cell line 6 (Fig. 3a, α-probe, lane 1; or β-probe, lane 1), whereas expression of both was clearly detectable in RNA from HPB-MLT (Fig. 3a, α-probe, lane 2; and β-probe, lane 2). Notably, Tγ mRNA was present in the

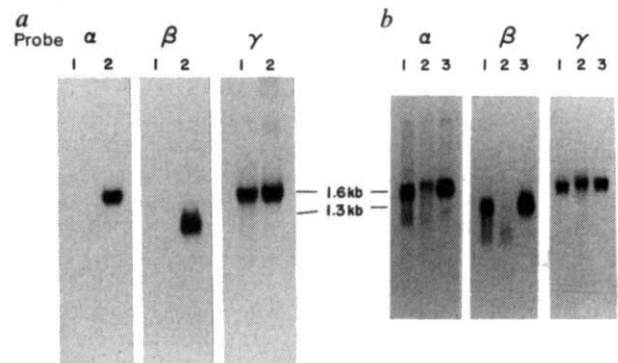


Fig. 3 Northern blot analysis of RNAs isolated from IDP2 cell lines using TCR α, TCR β and Tγ cDNA probes. **a**, Total RNA (15 µg) isolated from IDP2 cell line 6 (WT31⁻) (lane 1 for each probe) and from T-cell leukaemic line HPB-MLT (lane 2 for each probe) was fractionated on a 1.5% agarose gel containing 2.2 M formaldehyde, transferred to nitrocellulose and hybridized with TCR α, TCR β and Tγ probes. **b**, Total RNAs (2 µg) isolated from IDP2 cell line 5 (WT31⁺T3⁺) (lane 1 for each probe), IDP2 cell line 7 (88% WT31⁺T3⁺) (lane 2 for each probe) and HPB-MLT (lane 3 for each probe) were analysed as in **a**.

Methods. RNA preparation, electrophoresis, transfer to nitrocellulose and hybridization with ³²P-labelled, nick-translated probes (1–3 × 10⁸ c.p.m. µg⁻¹) were done as described previously⁴¹. The α-chain probes were the human cDNA clones pGA5 (ref. 8) in **a** and L17α (ref. 42) in **b**; the β-chain probes were human cDNA clones 12A1 (ref. 43) in **a** and L17β (ref. 43) in **b**. The γ-chain probe was an EcoRI–AccI fragment derived from human cDNA clone Tγ-1 (ref. 36). Radioactive bands were visualized by autoradiography using intensifying screens. In the experiment shown in **b**, all probes were labelled to almost identical specific activity, and identical exposure times were used.

WT31⁻T3⁺ cells at a level comparable to that in HPB-MLT (Fig. 3a, γ-probe, lanes 1 and 2). Thus, the WT31⁻βF1⁺T3⁺ lymphocytes lacked TCR α and β mRNA. Subsequent experiments on cell lines that were mostly WT31⁻T3⁺ corroborated this result. For example, Northern blot analysis of IDP2 cell line 7 (88% WT31⁺T3⁺) in comparison with IDP2 cell line 5 (WT31⁺T3⁺), as well as with HPB-MLT cells, revealed only a trace of TCR α or β mRNA in the 88% WT31⁻T3⁺ cells (con-

sistent with the 12% contamination with WT31⁺T3⁺ cells (Fig. 3b, lane 2 for each probe). Further, most of the β transcripts that could be detected were 1.0 and not 1.3 kilobases (kb) long and were probably nonfunctional³⁵. In contrast, the IDP2 cell line 5 (WT31⁺T3⁺) expressed levels of both RNA species which were comparable to those in HPB-MLT (Fig. 3b, lane 1 for each probe). However, like the WT31⁺T3⁺ line shown in Fig. 3a, both the WT31⁺T3⁺ and the WT31⁺T3⁺ lines showed levels of T γ RNA comparable to that in HPB-MLT (Fig. 3b, γ -probe).

Thus, the WT31⁺T3⁺ cells lacked α and β T-cell receptor mRNA (by Northern analysis) and α and β T-cell receptor proteins (by immunoprecipitation and FACS analysis). The presence of T γ mRNA in WT31⁺T3⁺ cells, while consistent with T γ protein expression, could not be taken as strong evidence for this, as many human cell lines that express T γ mRNA of normal size may express full-length transcripts that are out of frame because of defective joining of the V (variable) and J (joining) regions³⁶.

Non- α , non- β T3-associated molecules

We used chemical crosslinking to determine whether proteins analogous to the TCR $\alpha\beta$ molecules existed on the WT31⁺ β F1⁺T3⁺ cells. This technique has demonstrated the physical association of the TCR $\alpha\beta$ molecules with the T3 glycoprotein¹². The bifunctional, cleavable reagent dithiobis-succinimidyl propionate (DSP), was used to crosslink ¹²⁵I-labelled surface proteins of viable T lymphocytes. After crosslinking, the lymphocytes were solubilized in non-ionic detergent and immunoprecipitated with anti-T3 antibody. As expected, in the WT31⁺ β F1⁺T3⁺ lymphocytes the TCR α - and β -chains were found to be crosslinked to T3. For example, TCR $\alpha\beta$ molecules and T3 were found in anti-T3 or β F1 immunoprecipitates from crosslinked IDP1 cell line 2 (WT31⁺T3⁺) (Fig. 2a, lanes 4 and 6). However, despite the lack of reactivity with β F1 and the lack of TCR α or β mRNA, IDP1 cell line 3 (WT31⁺T3⁺) and IDP2 cell line 7 (88% WT31⁺T3⁺), both also expressed two protein subunits (55K and 40K) that specifically crosslinked to T3 (Fig. 2a, lane 10 and b, lane 6). Note that the mobilities of these T3-associated molecules were clearly different from those of the TCR α - and β -chains from WT31⁺T3⁺ cell lines (compare Fig. 2a, lanes 4 and 10, or b, lanes 5 and 6).

As IDP2 cell line 7 (88% WT31⁺T3⁺) contained 12% WT31⁺T3⁺ cells (accounting for the weak β F1 immunoprecipitates noted; Fig. 2b, lane 2), the lysate from these cells was precleared of TCR $\alpha\beta$ protein using β F1. After preclearing, no residual β F1-reactive material could be detected (Fig. 2b, lanes 8 and 11). When this β F1-precleared lysate from crosslinked cells was immunoprecipitated with anti-T3, 55K and 40K subunits were still detected (Fig. 3b, lane 12).

Because these WT31⁺ β F1⁺T3⁺ cell lines do not apparently express TCR α and β mRNAs, the molecules found specifically crosslinked to T3 on their cell surfaces cannot represent proteins encoded by the known TCR α or β genes. We therefore considered the possibility that one of these polypeptides represents the product of the rearranging T γ gene.

Isolation of T3-associated 55K subunit

cDNA clones representing the rearranging human T γ gene would encode a polypeptide with a predicted M_r of 40,000 (ref. 36). However, unlike the murine T γ gene, which does not reveal any N-linked glycosylation sites¹⁵, the human T γ gene has five potential sites for N-linked glycosylation, four of which are located in the constant region³⁶. As a T γ protein has not been isolated previously, it is unknown how many of these potential sites are used. However, a fully glycosylated human T γ protein may have a M_r of ~55,000. The heavy chain of the non- α , non- β T3-associated subunits identified on the WT31⁺ β F1⁺T3⁺ IDP1 and IDP2 cell lines described in the present report had a relative mobility of 55K on SDS-PAGE (Fig. 2a, b). To determine whether this T3-associated heavy chain was serologically cross-

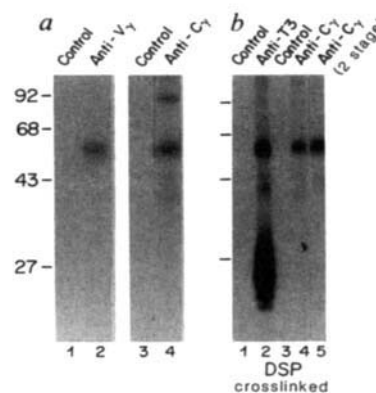


Fig. 4 Immunoprecipitations from IDP2 cell line 7 by anti-V γ and anti-C γ antisera. **a**, Triton X-100-solubilized, ¹²⁵I-labelled IDP2 cell line 7 (88% WT31⁺T3⁺) was denatured (see below) and then immunoprecipitated with NMS (lane 1) or normal rabbit serum (lane 3) and with anti-V γ antiserum (lane 2) or anti-C γ antiserum (lane 4). Note that a specific band was observed at M_r 55,000 in both the anti-V γ and anti-C γ immunoprecipitations. The additional band at M_r 90,000 was not reproducibly observed in the anti-C γ immunoprecipitations (see **b**). **b**, DSP-crosslinked native lysates (1% Triton-100) from ¹²⁵I-labelled IDP2 cell line 7 were immunoprecipitated with NMS (lane 1) or with anti-T3 antibody UCHT-1 (lane 2). Alternatively, the lysate was denatured (as described below) and immunoprecipitated with either normal rabbit serum (lane 3) or anti-C γ antiserum (lane 4). An additional aliquot of lysate was subjected to a two-stage immunoprecipitation: polypeptides were immunoprecipitated with UCHT-1, and were eluted from the immunoadsorbent under denaturing and reducing conditions, in order to break the DSP crosslink. Immunoprecipitation from this eluate was then performed using anti-C γ antiserum (lane 5).

Methods. ¹²⁵I-labelling, solubilization and immunoprecipitation were performed as described for Fig. 1. Native lysates (1% Triton-100) were denatured by adding SDS (final concentration 1%) and dithiothreitol (final concentration 2 mM) followed by heating the mixture for 5 min at 68 °C. After cooling, iodoacetamide was added (20 mM final concentration) and samples were diluted by adding 4 vol. of 1.5% Triton X-100 in Tris-buffered saline (pH 8). The initial immunoprecipitate in the experiment depicted in lane 5 of **b** was denatured and subsequently partially renatured in a similar manner²⁸. Samples were immunoprecipitated with 10 μ l of anti-C γ or anti-V γ antiserum, 1 μ g of UCHT-1 or 1 μ l of NMS or normal rabbit serum and analysed by SDS (10.5%)-PAGE under reducing conditions (5% 2-mercaptoethanol). Peptides corresponding to deduced V γ or C γ amino-acid sequences (residue numbers noted in the text) were synthesized on a Beckman 990 or an Applied Biosystems 430A peptide synthesizer using the method of Merrifield⁴⁴. Peptide purity was assessed by HPLC and peptide sequences were confirmed by amino-acid analysis. Peptides were coupled to keyhole limpet haemocyanin (KLH) at a ratio of 50 peptides per KLH molecule⁴⁵. Mice and rabbits were immunized with the V γ or C γ peptides, respectively. Animals were injected at 3-week intervals and the antisera screened for binding reactivity on peptide-KLH and peptide-bovine serum albumin conjugates to ascertain the presence of peptide-specific antibodies.

reactive with or identical to the T γ protein, antisera were raised against synthetic peptides representing, respectively, a stretch of 17 amino acids (residues 5–21) from the variable region (anti-V γ antiserum) and a stretch of 20 amino acids (residues 117–136) from the constant region (anti-C γ antiserum) of the T γ amino-acid sequence deduced from a human cDNA clone³⁶. Both the anti-C γ antiserum and the anti-V γ antiserum immunoprecipitated a molecule with a M_r of 55,000 from the denatured lysate of ¹²⁵I-labelled WT31⁺ β F1⁺T3⁺ cells (Fig. 4a, lanes 2 and 4). Such molecules could not be immunoprecipitated from lysates of ¹²⁵I-labelled HPB-MLT cells, which express only nonfunctional T γ mRNA³⁶ (data not shown).

To demonstrate that the 55K molecule immunoprecipitated by the anti- C_γ and anti- V_γ antisera was, in fact, the heavy subunit that crosslinked to T3, an additional experiment was performed (Fig. 4b). A sample of DSP-crosslinked lysate from the WT31 $^{-}$ β F1 $^{-}$ T3 $^{+}$ cells was first immunoprecipitated with anti-T3, again demonstrating the association of 55K and 40K subunits with T3 (Fig. 4b, lane 2). In parallel, another aliquot of the crosslinked lysate was immunoprecipitated with an anti-T3 monoclonal antibody, and the immunoprecipitated T3-crosslinked polypeptides were eluted from the immunoadsorbent, under denaturing and reducing conditions in order to break the DSP crosslink. This eluate was then re-precipitated with anti- C_γ antiserum; the 55K subunit that crosslinked to T3 was re-precipitated by the antiserum (Fig. 4b, lane 5), indicating that the 55K subunits defined by these two approaches were identical.

Conclusions

Framework monoclonal antibodies (β F1 and WT31) against the TCR $\alpha\beta$ molecules were used to identify and isolate a WT31 $^{-}$ β F1 $^{-}$ T3 $^{+}$ lymphocyte population from the PBL of two immunodeficiency patients. By the criteria of both immunoprecipitation analysis with framework monoclonal antibody and Northern blot analysis using TCR α - and β -specific cDNA probes, polyclonal human T-cell lines of this phenotype were shown to express neither TCR $\alpha\beta$ mRNA transcripts nor polypeptides. Nevertheless, chemical crosslinking studies using the cleavable DSP reagent revealed the existence of a protein complex associated with the T3 glycoprotein on the surface of these cells. The heavier of the two subunits that crosslinked to T3 (55K) was also immunoprecipitated by two different antisera, one generated against a 17-amino-acid synthetic peptide corresponding to a part of the variable region and another generated against a 20-amino-acid synthetic peptide corresponding to a part of the constant region of the deduced amino-acid sequence of a rearranged $T\gamma$ gene^{19,36}. Thus, it seems that the 55K protein is the $T\gamma$ protein encoded by the rearranging $T\gamma$ gene¹⁵ (or, less likely, a protein highly cross-reactive with it). Final proof of this, however, must await protein sequence determination and comparison with the deduced amino-acid sequences of rearranged $T\gamma$ cDNA clones. The 40K polypeptide appears to be a novel fourth T3-associated protein that we term T δ (Fig. 2a, b). The $T\gamma$ and T δ polypeptides may form a T3-associated heterodimeric structure ($T\gamma\delta$ -T3) on these cells that is analogous to the previously described T-cell receptor complex (TCR $\alpha\beta$ -T3). Alternatively, since the cell lines examined are polyclonal, the 55K and 40K polypeptides may occur as monomeric T3-associated subunits on individual T-cell clones.

The complex described here has several important characteristics that might be expected for a second T-cell receptor, that is, it is physically associated with T3 and one of the T3-associated chains is recognized by several anti- $T\gamma$ antisera. We do not know at present whether the $T\gamma$ and T δ components are distinct gene products, whether they are covalently linked via an inter-chain disulphide bond, whether they display clonal heterogeneity on T cells, or if ligands that react specifically with this protein complex are capable of triggering cell proliferation or factor production as occurs with the TCR $\alpha\beta$. However, in another study, human thymus-derived clones of the same phenotype as the IDP1 and IDP2 cell lines described here (β F1 $^{-}$ T3 $^{+}$) were stimulated to proliferate and to secrete IL-2 in response to anti-T3 antibodies (see page 179 of this issue⁴⁶). Thus, pending the outcome of peptide mapping and additional functional experiments, the T3-associated complex demonstrated here represents a likely candidate for a second T-cell receptor.

The function of the WT31 $^{-}$ β F1 $^{-}$ T3 $^{+}$ cells is unknown. $T\gamma$ mRNA expression is highest early in murine thymic ontogeny and declines thereafter, suggesting that the $T\gamma$ polypeptide may be functionally important only early in thymic ontogeny^{37,38}. Since the putative second T-cell receptor complex reported here has been identified in immunodeficiency patients, it may be expressed on cells arrested at an early stage of thymic ontogeny in these patients. For example, about 50% of the WT31 $^{-}$ β F1 $^{-}$ T3 $^{+}$ cells were T4 $^{-}$ T8 $^{-}$ T3 $^{+}$, which is similar in phenotype to an identified subpopulation of human thymocytes (ref. 39 and J. Allison and L. Lanier, personal communication). However, it is clear that the β F1 $^{-}$ T3 $^{+}$ phenotype occurs on a subpopulation of normal human PBL (Fig. 1c). These PBL may similarly express the non- α , non- β T3-associated complex described here. If so, this putative second T-cell receptor may occur on mature cells of a lineage which is separate, although related, to that of TCR $\alpha\beta$ -expressing T cells. Additional characterization of these novel T3-associated molecules and the cells that express them may further our understanding of T-cell ontogeny as well as mature T-cell function.

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Note added in proof: The 55K and 40K T3-associated polypeptides on IDP2 cell lines are not disulphide-linked.

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Infrared polarimetry of the nucleus of Centaurus A: the nearest blazar?

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As one of the nearest examples of an active galaxy, NGC5128 (Centaurus A) has been studied in detail over a wide range of wavelengths¹. The nucleus of the galaxy is seen clearly in the X-ray, radio and infrared, but is obscured in the optical by the prominent warped dust lane. We have made polarization observations of the infrared nucleus at wavelengths from 1.2 to 3.8 μm . We find that after correction for the polarization caused by the dust lane, and for dilution by starlight, the nucleus has a large intrinsic polarization of $\approx 9\%$ at position angle 147° . This position angle is perpendicular to the direction of the X-ray and radio jet. We interpret the polarized emission from the nucleus as synchrotron radiation from a region whose magnetic field is parallel to the jet direction. The properties of the Cen A nucleus are essentially identical to those of the much more luminous blazars¹⁷. This suggests that blazar-type activity extends over a very wide range in luminosity, and low-luminosity blazars may be common in elliptical galaxies.

Polarimetry and photometry of NGC5128 in the J(1.20 μm), H(1.64 μm) and K(2.19 μm) bands were obtained on 26 June 1985. Polarimetry in the L' band (3.8 μm) was obtained on 2 and 3 July 1985. All observations were made using the 3.9-m Anglo-Australian telescope and the Hatfield polarimeter². The position angles were calibrated using an internal calibration polarizer and standard stars for J, H and K, and an observation of the W33A infrared source³ for L'. The J, H and K observations were each obtained through apertures of nominal size 8, 4.5 and 2.25 arc s, centred on the peak 2.2- μm signal. Some vignetting was introduced by the wire grid polarizer, so the response was not flat over the full aperture. The seeing disk size was ~ 1 arc s (full width at half maximum) and the photometry was calibrated using standards observed through the same set of three apertures. The photometry and polarimetry results are given in Table 1.

Optical polarimetry of several points in the dust lane has been published by Elvius and Hall⁴. These observations show typical polarization position angles of $\sim 115^\circ$ near the nucleus. This polarization can be understood as being caused by absorption

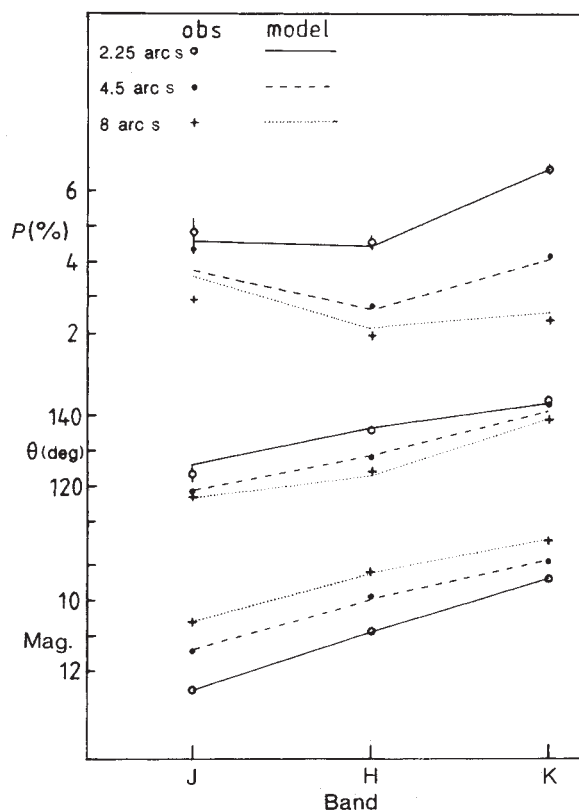


Fig. 1 Observations (points) of the polarization, position angle and magnitude of NGC5128 (Cen A) compared with the predictions (lines) of a model composed of a power-law polarized nucleus stellar component and dust lane polarization, as described in the text. Aperture sizes: 2.25 (○, —); 4.5 (●, ---) and 8 (+, ···) arc s.

by dust grains aligned by a magnetic field in the plane of the dust lane; thus, it can be expected to display the same wavelength dependence observed for interstellar polarization in our own Galaxy⁵, and decrease into the infrared. The polarizations we observe in the J band in the largest aperture show approximately the same position angle as this dust lane component, but as we go to smaller apertures and longer wavelengths we find increasing polarizations, and a tendency for the position angle to swing to larger values. This effect can be readily understood if the very red point-source nucleus⁶ has its own intrinsic polarization with a position angle different from that caused by the dust lane. Smaller apertures and longer wavelengths give increasing contributions of the nucleus relative to the stellar component of the galaxy, and thus a total polarization and position angle closer to that of the nucleus.

Using the multi-aperture observations we can derive the flux and polarization of the annuli between 2.25 and 8 arc s, and between 4.5 and 8 arc s. Although there are differences between the two annuli, the K-band polarizations are very small ($\sim 0.5\%$, compared with the 6.63% measured in the smallest aperture), confirming that the polarization is predominantly due to the nucleus itself. The light contained within the smallest aperture will be that from the polarized nucleus itself, and the contribution from starlight. We can reasonably assume that the starlight within the smallest aperture has the same colours and polarization as those observed for the annuli. We can set reasonable limits on the relative contributions to the flux, by requiring that the nuclear flux at J must be greater than zero, but must not be so large that the surface brightness of the stellar component would decrease inwards.

Table 1 Polarimetry and photometry of NGC5128

Aperture (arc s)	Band	Magnitude	P (%)	θ (deg)
2.25	J	12.60	4.94 ± 0.42	123.9 ± 2.3
	H	10.96	4.63 ± 0.20	136.4 ± 1.3
	K	9.60	6.63 ± 0.14	144.8 ± 0.3
4.5	J	11.48	4.43 ± 0.14	119.3 ± 1.2
	H	9.95	2.80 ± 0.08	128.6 ± 0.8
	K	8.98	4.22 ± 0.10	144.4 ± 1.4
	L'		6.1 ± 0.9	146 ± 6
8.0	J	10.67	2.99 ± 0.14	118.4 ± 0.8
	H	9.24	1.94 ± 0.06	124.9 ± 0.8
	K	8.32	2.39 ± 0.11	139.6 ± 1.5

Using the above procedure, we find a range of values of the polarization of the nucleus. The K polarization must be in the range 8.8–11.1%, the H polarization in the range 7.2–15.4%, and the J polarization must be $>7.1\%$. The smallest values correspond to the largest flux contributions from the nucleus. Thus, this analysis suggests a polarization which is either relatively flat with wavelength or increases to the blue.

We have obtained a good fit to the data with a model consisting of the following components (see Fig. 1 and Table 2): (1) A power-law nucleus with a wavelength-independent polarization of 9% at position angle 147° , spectral index 5.3, and J-band flux of 3.0 mJy. (2) A stellar component with spatial flux distribution adjusted to fit the J photometry, and colours equal to those observed for the 4.5–8 arcs annulus. (3) A polarization due to the dust lane, applied to both of the above components, following a Serkowski law with $P_{\max} = 7\%$, $\lambda_{\max} = 0.55 \mu\text{m}$ and position angle $= 115^\circ$.

The fit is generally good. The discrepancies in the J-band polarization are probably due to the patchy nature of the dust lane⁷, causing its polarization to change over the spatial scale of the observations. Slight discrepancies in the fit to the flux indicate that a power law is not an accurate representation of the spectrum. A reddened power law may fit the data better, but the degree of reddening appropriate for the nucleus is uncertain. The L' polarization is a little lower than predicted by the model, perhaps indicating some additional source of dilution (such as thermal radiation from the dust lane as seen at $10 \mu\text{m}$ (ref. 8)).

There is evidence that the nucleus of Cen A has a much higher extinction than the A_V of 5–6 mag (ref. 9) due to the dust lane. The silicate optical depth implies $A_V = 22$ mag (ref. 10), and the hydrogen column density derived from X-ray absorption implies $A_V = 45$ mag (ref. 11). However, the wavelength dependence which we observe for the polarization of the nucleus does not rise to the blue steeply enough for the polarization to be due to dichroic absorption in this thick dust shell. A small contribution to the polarization arising from the dust shell, in addition to a flatter intrinsic polarization from the nucleus, cannot be ruled out.

The position angle of the X-ray jet is $53 \pm 1^\circ$ (ref. 11), and that of the radio jet is $55 \pm 7^\circ$ (ref. 12). The polarization position angle we observe for the nucleus is almost exactly perpendicular to these jet position angles and suggests that the polarized infrared radiation is associated with the inner jet. Antonucci¹³ has noted other cases of radio galaxies with polarization perpendicular to the radio structure.

Models proposed for the nucleus of Cen A^{14–16} explain its infrared flux as synchrotron radiation, with inverse Compton scattering of the infrared photons giving rise to X-ray emission. Our observation of high polarization for the infrared nucleus provides strong support for such synchrotron models. The observed position angle then indicates that the magnetic field must be aligned predominantly along the jet direction. The only other plausible origin for the infrared nucleus is thermal re-radiation by dust grains, which could produce a high polarization if the emitting grains has a high degree of alignment. However, Grasdalén and Joyce¹⁰ have considered this thermal

Table 2 Components of flux within a 2.25 arc s aperture

Band	Flux of nucleus (mJy)	Flux of Galaxy (mJy)	Total flux (mJy)	Magnitude	P (%)	θ (deg)
J	3.0	12.0	15.0	12.60	4.57	125.4
H	15.7	25.6	41.3	10.99	4.49	136.6
K	74.5	32.2	106.7	9.46	6.66	144.0

re-radiation model and find that it cannot account for the observed infrared flux.

The class of blazars¹⁷ includes the BL Lac objects and the highly polarized quasars. Such objects are considered to be normally found in the nuclei of giant elliptical galaxies, possess compact radio sources with flat or inverted spectra and highly polarized, smooth optical/infrared continua, and are subject to violent variability. The observation of a highly polarized infrared continuum shows that all of these properties are shared by the nucleus of Cen A, with the possible exception of violent variability. There is no evidence for variability of the infrared nucleus of Cen A, although this may be due to insufficient observations. The existence of variability of the Cen A nucleus at X-ray wavelengths is well established¹¹. Studies of BL Lac objects show that the most violently variable objects are those of highest luminosity, with low-luminosity objects tending to show less variability and polarization with preferred position angles¹⁷. Similar effects can be seen in individual objects, such as BL Lac, which shows well-defined position angles when it is faint¹⁸. Thus, the absence of violent variability in Cen A, which is substantially less luminous than a typical blazar, need not preclude it being closely related to such objects. BL Lac objects typically show polarization which is either flat with wavelength or decreases to the red^{19,20}, consistent with what we see in Cen A.

The luminosity of the Cen A nucleus in the 1–2- μm region is uncertain due to the unknown extinction, but is probably $<10^{42} \text{ erg s}^{-1}$. The luminosities of BL Lac objects²¹ in the same spectral region range from 10^{44} to $10^{47} \text{ erg s}^{-1}$. However, the existence of blazar activity at such low luminosities is not surprising. Individual blazars (for example, 3C279²²) can vary by as much as a factor of 500. This range presumably reflects changes in the accretion rate onto a massive black hole. It is also thought that relativistic beaming may result in enhancements in apparent luminosity of up to a factor of 100 in the most luminous objects²³. Thus, a blazar which is currently at a low accretion rate, and is not beamed towards us, could have a luminosity lower by a factor of $\geq 50,000$ than that of the most luminous examples. Cen A may well be such an object. Other probable examples of low-luminosity blazar activity are IC5063, which has an infrared nucleus²⁴ which we have recently found to be highly polarized, and NGC1052²⁵. Such objects may be relatively common, but in galaxies at distances much greater than that of Cen A they would become very difficult to find, because the stellar flux from the galaxy would dominate over the polarized non-thermal emission from the nucleus.

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The soft γ -ray burst GB790107

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Nearly all of the known γ -ray bursts (GRBs), when observed over the energy range ~ 30 keV to 1 MeV, have intensity spectra that can be described in terms of several-hundred-keV exponential functions. The Venera 11 and Venera 12 KONUS data indicate that, in addition to these 'normal' GRBs, there might exist a separate class of short, soft events^{1,2}. There can be little doubt that bursts with spectra that seem anomalously soft (e-folding energy ≤ 30 keV) do exist, and that the most convincing known examples—GB790305b (the famous 5 March burst, including its follow-on events), GB790324 (and its follow-ons) and GB790930—generally have atypically short durations of ~ 0.1 – 0.25 s. (Three of the 11 GB790305b follow-on events were longer³.) However, because most GRB instruments were designed primarily for observations above ~ 100 keV, events with very soft spectra have not been well enough studied to be classified unambiguously. Here we present the results of fortuitous detections of GB790107 (determined in ref. 2 to have a soft spectrum) with instrumentation better suited for the study of such bursts. Above 20 keV the spectrum is indeed much softer than any other cosmic event observed through the apertures of these experiments, and does not appear to belong to the general GRB spectral distribution. However, in the 5–15-keV range the spectrum is very flat. Statistical arguments indicate that soft-spectrum events might belong to a disk population, but this is not a strong conclusion.

By virtue of their ~ 20 -keV thresholds, the KONUS experiments have been, until now, the only sources of data on bursts (apart from the 5 March event) with spectra that might be anomalously soft. These observations, although valuable, have significant limitations imposed by the KONUS 20-keV thresholds and 4-s accumulation times for spectral measurements. Clearly, information at lower energies and with better time resolution is required for the study of events having typical energies of ≤ 30 keV and durations of ~ 0.2 s. However, experiments with appreciably lower energy thresholds must be collimated to avoid excessive background rates caused by the diffuse X-ray background. Thus, we were fortunate to have simultaneously observed GB790107 down to 5 keV with 0.5-s spectral time resolution with a collimated instrument on the International Cometary Explorer (ICE) spacecraft⁴, and down to 13 keV with 0.25-s resolution with a collimated instrument on Prognost 7 (P7)⁵. Unfortunately, however, GB790107 was not observable by most GRB instrumentation, due to low instrumental sensitivities below 30 keV. Thus, the detection in the near future of additional similar events could perhaps best be accomplished by examining the 5–100-keV ICE real-time data record, which spans ~ 6 yr at high duty cycle, with good spatial coverage and 0.5-s time resolution. (As mentioned below, we have already searched the equivalent of a 70-day, 100%-duty-cycle block of these data.)

Because of the brevity of GB790107 and the fact that its spectrum was too soft to trigger the high time-resolution memories of most GRB experiments, a well-resolved time history (energy-integrated) was obtained only by the KONUS experiment¹ (Fig. 1 inset). The outburst had a duration of ~ 0.2 s and

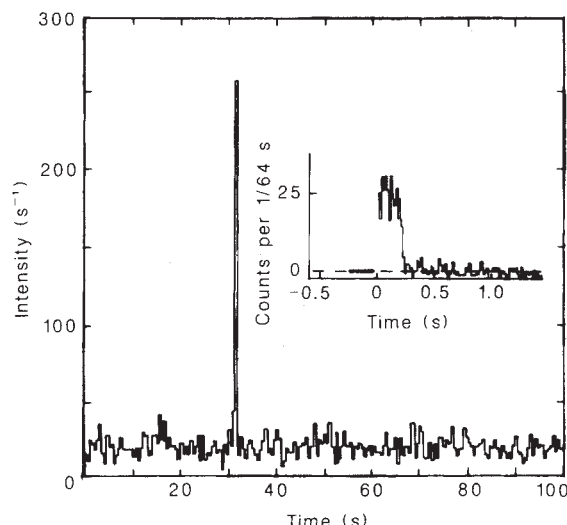


Fig. 1 Time histories of GB790107. The inset is the KONUS energy-integrated time history, and the main plot is the ICE 25–40-keV response. This was one of the most intense cosmic events ever seen in ICE data in this band, but its flux above ~ 100 keV was unobservable at current instrumental sensitivities.

a rather flat-topped profile. The ICE response in the 25–40-keV band (Fig. 1) is one of the most intense ever recorded by that instrument from a cosmic source. The P7 and ICE data records do not resolve the main spike, but the continuous ICE time coverage with 0.5-s resolution allows one to search for slow structure, such as '5 March-like' pulsations. As can be seen from Fig. 1, there are no indications of any such structure, although appropriately scaled-down GB790305b pulsations would not be observable.

Figure 2 shows the GB790107 spectral data. Because of the uncertainty in the source location, the absolute normalizations of the spectra measured by the collimated instruments on P7 and ICE are uncertain by about $\pm 25\%$. The normalizations used in the figure assume the highest source exposures that are consistent with the time-of-arrival error box. Also, a spacecraft strut that is sometimes in the ICE field of view could give rise to an additional 25% systematic error below 15 keV for this event. Our conclusions are not affected by either the normalization or the 'strut-absorption' uncertainties. Above ~ 30 keV the spectrum is extremely steep for a GRB, corresponding to a temperature of ~ 10 keV if fit by a Planck function or ~ 30 keV if fit by optically thin thermal bremsstrahlung. (The GB790305b and GB790324 follow-on events had similar temperatures².) However, our data show that the steepness does not extend to lower energies; the 5–7-keV upper limit is comparable to the measured intensity at 30 keV. The high-energy 'tail' which seems to be present in the KONUS spectrum was not detected by the other experiments, even though their sensitivities were adequate. Even without the tail, simple models can reproduce the data only if some means of explaining the lack of low-energy photons is provided. It may be noteworthy that the thermal cyclotron model, with its lack of emission below the first harmonic, can provide a 'natural' low-energy cutoff if the magnetic field in the emission region is $\sim 10^{12}$ G.

The time history and spectral information give some insight concerning whether GB790107 belongs to, or is closely related to, any other known class of high-energy transient. First, we note that the only other cosmic events having a comparable 25–40-keV intensity in ICE records (GB781104b, GB790305b, GB820801 and GB840304) were among the most powerful GRBs ever observed at energies > 100 keV. At these 'typical' GRB energies, GB790107 is at least ~ 2 orders of magnitude weaker than the aforementioned strongest bursts. None of the ~ 100 other known ICE events had an intensity in the 25–40-keV band

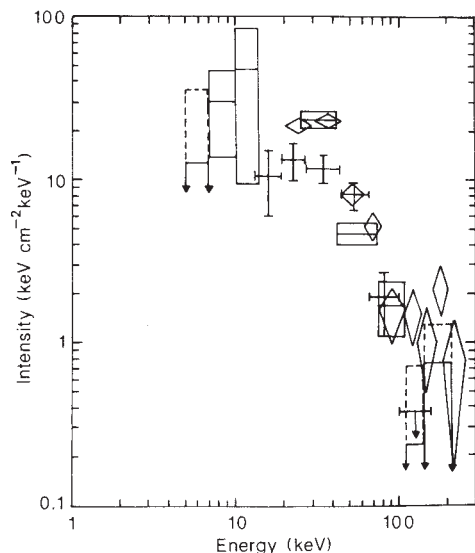


Fig. 2 GB790107 spectra. The plot is non-standard for γ -ray bursts in that it is an intensity spectrum rather than a number spectrum. This representation generates a clearer picture of the very steep GB790107 spectrum. Rectangles, ICE; crosses, P7; diamonds, KONUS data. The upper limits are shown at 1σ (solid lines) and, for ICE, 2σ (dashed lines) confidence levels. The integration times for the spectra are 0.5 s for ICE and P7, and 4 s for KONUS. The burst duration was 0.2 s.

greater than one-quarter that of GB790107. It is therefore difficult, but not impossible, to believe that GB790107 is a member of the 'classical' GRB population. Furthermore, its short duration and high (>10 keV) temperature set it well apart from X-ray bursters, and its brevity and lack of a prominent gradual X-ray component differentiate it from solar flares. The only events both spectrally and temporally similar to GB790107 are the follow-on events associated with GB790305b and GB790324. (GB790107 does resemble spectrally the impulsive components of some solar flares, and a weak, gradual component could have gone undetected. This might be a clue to the emission mechanism.)

The GB790107 localization information is derived from wave-front arrival times at Venera 11, P7 and ICE, and from the responses of the collimated ICE and P7 instruments. The error box (more properly, the 'error arc') is defined by the intersection of the following areas: (1) the P7/KONUS arrival-time annulus of radius $33.475 \pm 0.012^\circ$ centred at $(\alpha, \delta)_{1950} = 15 \text{ h } 43 \text{ min } 53.6 \text{ s}, -18^\circ 04' 53''$; (2) an 8° full-width band, centred on the ecliptic plane, inferred from the relative ICE response; and (3) a region $\sim 15^\circ$ by 20° , centred near $(\alpha, \delta) = (18 \text{ h } 20 \text{ min}, -25^\circ)$, determined from the P7 response. The intersection of these regions is a curved strip, 8° by 0.024° , whose midpoint is at $(\alpha, \delta)_{1950} = (18 \text{ h } 05 \text{ min } 36.8 \text{ s}, -23^\circ 26' 22'')$ or $(l_2, b_2) = (7.36, -1.71)$. A search through catalogues of flare stars, pulsars and high-energy emitters ($\sim 25,000$ objects) revealed two in the box. These were the pulsar PSR1804-27 (ref. 6) and V2 57, an early-type star with emission lines⁷. In this region of the sky there are several catalogued objects per square degree, so chance coincidences are not unlikely. Also, due to its own location uncertainty, the pulsar may not be in the GRB error box.

Perhaps the most significant question about the error box concerns what its proximity to the galactic centre (GC) tells us about the spatial distribution of this type of event. The following additional facts are relevant: (1) The localized events that are spectrally and temporally most similar to GB790107 (that is, GB790305b and GB790324) are located at $(l_2, b_2) = (275, -33)$ and $(47, 4)$. (2) GB790930, the most certain (in our judgment) other identification of a short event with a soft spectrum, did not produce a response in ICE, and was therefore at least 30°

from the GC (but not necessarily out of the plane). (3) If the flat top of the GB790107 time history is caused by the Eddington limit, then the distance to the source is ~ 1 kpc, and the low longitude is due to chance. (4) The ICE experiment, whose field of view always includes most of the GC region, has not observed a single additional cosmic event with a comparably soft spectrum. (Our searches have included lists of suspected events^{1,2,8,9}, based on their short durations or published spectra, as well as an arbitrary 70-day-equivalent data block. The other ~ 10 short events observed by ICE had hard spectra.) These facts all imply that there is not much concentration in galactic longitude, although the three known locations are at low latitudes. A disk distribution may thus be indicated but this is not a strong conclusion.

There remain many basic questions concerning the spatial, temporal (both frequency of occurrence and time history) and spectral distributions of soft bursts. Until we obtain some of the answers (or the basic GRB mechanism becomes well understood) we cannot state definitively that GB790107 and other soft events constitute a true 'class', as opposed to being merely 'extreme' examples of GRBs.

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Note added in proof: We have recently discovered multiple (perhaps >50) recurrences of GB790107 in 1983. This confirms its similarity to the March 1979 repeating sources, and strengthens the interpretation that these objects are members of a distinct 'class'.

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Evidence for an asymptotic lower limit to the surface dipole magnetic field strengths of neutron stars

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The discovery of a second millisecond binary radio pulsar¹, PSR1855+09 (period $P=5$ ms), with a relatively wide circular orbit and low mass function indicates that the incidence of such systems (see also refs 2, 3) among the galactic radio pulsar population is $\sim 4 \times 10^{-3}$. The generally accepted model for the origin of these systems is that they have descended from low-mass X-ray binaries in which a weakly magnetized neutron star³ was spun-up by the accretion of matter from an evolved low-mass ($\leq 1.2 M_\odot$) companion star, which has now ended its life as a

helium white dwarf³⁻⁸. We show here that, based on the incidence of the progenitor low-mass X-ray binaries (LMXBs), the observed incidence of millisecond binary radio pulsars can be explained only if, at their present low field strength of $\sim 10^9$ G, the decay timescale of neutron-star surface magnetic dipole moments is $\geq 10^9$ yr. This possibility has been advanced by Kulkarni⁹, who estimates, based on the optical detection of the counterpart of the weak-field binary radio pulsar PSR0655+64, that this pulsar is old ($> 5 \times 10^8$ yr) and hence that the magnetic field decay timescale becomes very large ($> 10^9$ yr) for field strengths $\leq 10^{10}$ G. Our findings provide independent support for this possibility.

The progenitor LMXBs, in which the companion stars are evolved, are strong sources, of X-ray luminosity $L_X \approx (0.2-1) \times 10^{38}$ erg s⁻¹, such as the strong galactic bulge sources, Sco X-1 and Cyg X-2 (ref. 10). There are at most 30-50 such sources in the Galaxy¹¹, of which about a dozen are located in the galactic bulge. The typical X-ray lifetime of these systems, based on their accretion rates ($\dot{M}$) of $\sim (0.2-1) \times 10^{-8} M_\odot$ yr⁻¹ and companion mass $\sim 1 M_\odot$, is $\sim 2 \times 10^8$ yr (ref. 12). This implies a formation rate of these luminous LMXBs of at most $(1.5-2.5) \times 10^{-7}$ yr⁻¹. Assuming all such systems to leave millisecond binary radio pulsars, the number of millisecond binary radio pulsars formed during the $\sim 1.5 \times 10^{10}$ yr lifetime of the Galaxy is $\sim 3 \times 10^3$ (in a steady state, their formation rate will be the same as that of the luminous LMXBs).

As pointed out by Taam and van den Heuvel¹³, the presently available data on magnetic field strengths of neutron stars in binaries are consistent with the hypothesis that neutron stars are born with surface dipole field strengths $B_s \approx 10^{12}-10^{13}$ G, which decay exponentially on a timescale of $\sim 5 \times 10^6$ yr for $\sim 7-10$ e-foldings, that is, down to at least 10^9-10^{10} G. In order to become a millisecond pulsar, that is, to reach $P_{\text{rot}} \leq 5$ ms by spin-up, B_s should have decayed to $< 3 \times 10^9$ G before the end of the accretion spin-up phase, as otherwise, even at $\dot{M} = \dot{M}_{\text{Edd}}$ (the accretion rate corresponding to the Eddington limit), such a short period cannot be reached (see refs 8, 13, references therein, and equation (1), below). If the surface dipole fields of such pulsars were to keep decaying beyond this value on a timescale of $\sim 5 \times 10^6$ yr, the field strengths of these millisecond pulsars would drop below 5×10^7 G within $\sim 2 \times 10^7$ yr after the end of the accretion spin-up phase, rendering them virtually unobservable as pulsars (no known radio pulsar has a field strength of $< 4.5 \times 10^8$ G). With the formation rate in the Galaxy derived above, this maximum lifetime of $\sim 2 \times 10^7$ yr as a millisecond pulsar would imply that, at any time, at most only three to five millisecond binary radio pulsars would be observable in the entire Galaxy. The total number of active radio pulsars in the Galaxy is estimated to be $(1-5) \times 10^5$ (ref. 14), leading to an expected incidence of millisecond binary pulsars among radio pulsars of at most 6×10^{-6} to 6×10^{-5} .

In contrast with the above estimate, among the ~ 500 known radio pulsars, there are already two millisecond binary pulsars (1953+29 and 1855+09). The distances of these two systems as inferred from their dispersion measures (~ 3 and 0.3 kpc, respectively) are in the same range as those of most of the known radio pulsars. The incidence of binary millisecond pulsars in the general pulsar population is therefore likely to be of the same order as their incidence among the known sample of pulsars, which is $\sim 4 \times 10^{-3}$. This is $\sim 10^2-10^3$ times higher than expected under the assumption that at $B_s < 3 \times 10^9$ G the fields continue to decay on a timescale of $\sim 5 \times 10^6$ yr.

Apparently, the latter assumption cannot be correct. The only solution to this problem seems to be that, once the surface dipole field strength has decreased to $\leq 3 \times 10^9$ G, the decay timescale of the field has increased by a factor of 10^2-10^3 over that at $B_s \approx 10^{12}-10^{13}$ G. Therefore, at $B_s < 3 \times 10^9$ G the field decay timescale must thus be at least $\geq 10^9$ yr, and possibly $> 10^{10}$ yr. As the fields of the binary radio pulsars PSR1913+16 and PSR0655+64 are already close to this boundary (2.2×10^{10} and 1.3×10^{10} G, respectively; see ref. 13), they may be expected to

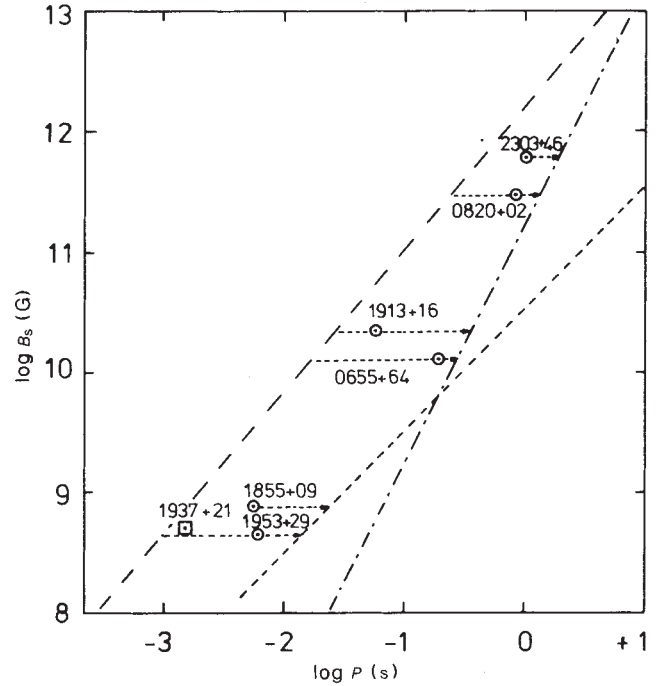


Fig. 1 B_s versus P diagram, showing the positions of the six binary pulsars with known $\dot{P}$ values, together with the 'spin-up line', $P_{\text{min}}(\dot{M}_{\text{Edd}}, B_s)$, given by equation (1) (—), the death-line³ (---) and the P_f line (the final period reached after a Hubble time, if spin-down proceeds at constant B_s) (— · —). The figure shows that spun-up pulsars with $B_s < 6.10^9$ G need longer than a Hubble time to reach the deathline (assuming evolution at constant B_s).

decay much more slowly than the fields of young, strong-field pulsars. These pulsars may therefore be considerably older than their formal ages, calculated with a field-decay timescale of $\sim 5 \times 10^6$ yr, would indicate. This gives independent support for the high age ($\geq 10^9$ yr) of PSR0655+64 as inferred from the faintness of the optical counterpart of its companion⁹.

With an incidence of at least $\sim 4 \times 10^{-3}$ among the $(1-5) \times 10^5$ radio pulsars in the Galaxy, the total number of active binary millisecond pulsars is expected to be between at least ~ 400 and $2,000$. Among these, the fraction in globular clusters is expected to be an order of magnitude less than one would infer on the basis of the incidence of LMXBs in such clusters. This is because in a globular cluster a neutron star in a binary may many times capture a fresh companion by means of an exchange collision¹⁵⁻¹⁷, thus lengthening its X-ray lifetime by an order of magnitude. Based on the number of strong sources in globular clusters (~ 10), this yields a formation rate of millisecond pulsars of 5×10^{-9} yr⁻¹ in the globular cluster population, which, with a lifetime of 10^9-10^{10} yr yields $\sim 5-50$ active binary millisecond pulsars. A pulsar survey of globular clusters would therefore be valuable.

Upper limits to the ages of the six binary radio pulsars with known period derivative ($\dot{P}$) can be obtained by assuming that they have all been spun up during the accretion phase to the shortest possible spin period $P_{\text{min}}(\dot{M}, B_s)$, and have subsequently spun down as radio pulsars to their present pulse periods, at a constant value of B_s . P_{min} is given by^{8,13,18}

$$P_{\text{min}} = (1.9 \text{ ms}) B_9^{6/7} (M/1.4 M_\odot)^{-5/7} (\dot{M}/\dot{M}_{\text{Edd}})^{-3/7} R_6^{18/7} \quad (1)$$

where B_9 is the neutron-star magnetic field in units of 10^9 G, M is the mass of the neutron star, $\dot{M}$ the accretion rate and R_6 the neutron-star radius (in units of 10^6 cm). With $\dot{M} = \dot{M}_{\text{Edd}}$, $M = 1.4 M_\odot$ and $R_6 = 1$, the values of P_{min} in Table 1 are obtained. The spin-down time from this period to the presently observed

Table 1 Observed and calculated parameters for the six binary pulsars with known $\dot{P}$ -values²⁶, for the case of evolution at constant magnetic field

Binary pulsar	P (s)	B_s (10^9 G)	$P_{\min}$ (ms)	Max age (10^9 yr)	P_f (s)	$P_{\text{t.o.}}$ (s)	$\Delta t_{\text{t.o.}}$ (10^9 yr)
PSR0655+64	0.196	12.6	16.7	3.9	0.38	0.30	5.3
PSR0820+02	0.865	300	252	0.124	9.1	1.36	0.2
PSR1855+09	0.0054	0.8	1.57	0.68	0.025	0.07	—
PSR1913+16	0.059	22	24.8	0.097	0.67	0.36	4.2
PSR1953+29	0.0061	0.45	1.0	2.9	0.014	0.05	—
PSR2303+46	1.066	660	500	0.033	Very long	1.8	0.08

Calculations assume $M = 1.4 M_{\odot}$, $I_{45} = R_6 = 1$.

spin period, at constant B_s , is then obtained by integrating the spin-down equation²:

$$(P\dot{P}) = R_6^6 I_{45}^{-1} B_s^2 / (1.024 \times 10^{39}) \text{ s} \quad (2)$$

where I_{45} is the moment of inertia of the neutron star in units of 10^{45} g cm^2 . The resulting ages, listed in Table 1, are upper limits to the ages of these pulsars, because if B_s at the end of the spin-up phase was larger than at present, their true ages will be smaller.

Table 1 shows that PSR2303+46, PSR0820+02 and PSR1913+16 cannot be older than $\sim 0.33 \times 10^8$, 1.2×10^8 and 1.0×10^8 yr, respectively. On the other hand, PSR1855+09, PSR0655+64 and PSR1953+29 might be as old as 0.7×10^9 , 4×10^9 and 3×10^9 yr.

Table 1 also lists the final spin period, P_f , attained by each pulsar, one Hubble time ($\sim 1.5 \times 10^{10}$ yr) after its birth at $P = P_{\min}$, if the surface dipole field remains constant. These periods are derived from the integration of equation (2), which yields

$$P(t)^2 = P_0^2 + 1.95 \times 10^{-21} R_6^6 I_{45}^{-1} B_s^2 t \quad (3)$$

Also shown in Table 1, as well as in Fig. 1, are the turn-off periods, $P_{\text{t.o.}}$ of these pulsars, corresponding to the 'deathline' (see ref. 3), for evolution at constant B_s , and the lifetimes, $\Delta t_{\text{t.o.}}$, until reaching the deathline.

Table 1 and Fig. 1 show that the four binary pulsars with the largest B_s values will reach the deathline within one Hubble time. If their fields were to decay, their final periods would be shorter and they might remain observable for a Hubble time. (Note, however, that PSR1913+16 will coalesce with its companion due to gravitational radiation losses within $\sim 3 \times 10^8$ yr, long before it reaches the deathline¹⁹.) On the other hand, PSR1855+09 and PSR1953+29 do not reach the deathline within a Hubble time. (The same holds for the 1.55-s single pulsar PSR1937+21, which has $B_s = 5 \times 10^8$ G, and is also expected to have had a history of spin-up in a binary system, although of an entirely different type (see refs 13, 20, 21, and references therein).) These three pulsars may therefore 'live forever'. These conclusions are in agreement with and provide added support for the findings of Kulkarni⁹.

As to the interpretation of the 'asymptotic' field strength: according to the work of Ruderman and co-workers²² the crustal currents that support the huge exterior magnetic field ($\sim 10^{12}$ – 10^{13} G) of a newborn neutron star decay on a timescale of $\sim 10^7$ yr by ohmic dissipation. In contrast, any residual magnetic field arising from core currents will have a much longer life—possibly longer than a Hubble time—due to proton superconductivity in the core (see also refs 23, 24). In this interpretation, the resulting asymptotic field strength is not expected to be the same for all neutron stars, as the strength of the core currents, frozen in at the time of collapse, will depend on the individual history of the parent star (for example, its rotation and magnetic field).

An alternative, purely phenomenological interpretation, consistent with the observations listed in Table 1, is that magnetic fields of non-accreting neutron stars do not decay at all (a

possibility suggested by Kundt²⁵), but that accretion gradually weakens the field. Indeed, in the long-lived ($\geq 10^8$ yr) LMXBs which produced the binary millisecond pulsars PSR1953+29 and PSR1855+09, the neutron stars are expected to have accreted $> 0.1 M_{\odot}$, and possibly as much as $1.0 M_{\odot}$ (refs 7, 13). The same holds for the millisecond pulsar PSR1937+21 (refs 8, 18). On the other hand, PSR2303+46, PSR1913+16 and PSR0655+46, which have much stronger fields than the three millisecond pulsars, are expected to have accreted $\leq 0.01 M_{\odot}$, as they have descended from massive X-ray binaries in which the accretion phase lasted $< 10^6$ yr (refs 7, 8, 13). The same holds for PSR0820+02, where, again, the accretion phase lasted less than a few million years^{7,8,13}.

We cannot offer an explanation for a possible relationship between field decay and amount of matter accreted. It seems conceivable, however, that both the crustal and interior structure of a newborn neutron star that formed in the liquid state at $T > 10^{10}$ K and subsequently cooled and underwent crustal freezing²², is quite different from that of a neutron star that subsequently underwent considerable accretion at relatively low temperatures ($T < 10^8$ K).

It is not now possible to decide which of the two alternative interpretations of the asymptotic field behaviour is the correct one, although the first-mentioned one appears most plausible to us.

After submission of this paper we received a preprint by Bhattacharya and Srinivasan²⁷, who independently, and from a somewhat different viewpoint, also reached the conclusion that the decay timescale of the magnetic fields of binary millisecond pulsars must be $\sim 10^9$ yr.

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Inhibition of convective collapse of solar magnetic flux tubes by radiative diffusion

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Interaction of convection with a magnetic field leads to an intermittent distribution of magnetic flux¹. Such a process operating on the solar surface can lead to 'equipartition' fields of 700 G (ref. 2). These fields are further prone to a convective instability and eventually collapse to kilogauss intensity³⁻⁵. I show here that radiative diffusion can inhibit this collapse to a varying degree, depending on the field strength and the thickness of the flux elements. As a consequence, one would expect the field strength of the photospheric magnetic flux elements to depend on their sizes. It is shown that at one end of such a distribution there would be kilogauss tubes with small dispersion in field strength and large dispersion in size. At the other extreme of the spectrum would be thin tubes of fairly constant size but with a wide range in field strength, from kilogauss intensities to the equipartition values of 700 G. High-resolution observations from space-borne telescopes should reveal the existence of the latter variety of tubes.

An approximate but effective method of examining the stability of magnetic equilibria resembling solar flux tubes is to use the slender-flux-tube equations⁶. The linearized equations for slender, radiating, optically thin flux tubes have been considered by Webb and Roberts⁷ in the context of radiative damping of tube waves. One can use the same equations for optically thick tubes by re-defining the radiative relaxation time τ_R of ref. 7 as $\tau_R = 4\chi/r_0^2$ where χ is the radiative diffusivity and r_0 is the cross-sectional radius of the tube⁸. For mathematical simplicity and to focus attention on the physics of the problem, we assume isothermal stratification in the tube. The imposition of boundary conditions of vanishing velocity perturbation at the two ends of the tube on equation (12) of ref. 7 (after correcting a misprint in that equation by replacing Λ^2 with Λ_0) leads to the following dispersion relation:

$$\tilde{\sigma}^3 + a_2\tilde{\sigma}^2 + a_1\tilde{\sigma} + a_0 = 0 \quad (1)$$

where $a_0 = \epsilon b_0/\delta$, $a_1 = -\{(1-\gamma)(1+\beta_0)/2 - \gamma b_0\}/\delta$, $a_2 = \epsilon(1+\beta_0/2)/\delta$, $\delta = (1+\gamma\beta_0/2)$, $b_0 = 1/16 + \pi^2 n^2 \Lambda_0^2/d^2$, $\epsilon = \tau_D/\tau_R$, $\tilde{\sigma} = \sigma\tau_D$ and $\tau_D = (\Lambda_0/g)^{1/2}$. Here, σ is the complex frequency of the perturbation, assumed to grow in time as $e^{\sigma t}$, β_0 is the ratio of gas pressure to magnetic pressure in the tube, n is the harmonic of the perturbation, d is the length of the tube, Λ_0 is the isothermal scale height of the atmosphere and g is the acceleration due to gravity. To simulate a superadiabatic temperature gradient (necessary for convective collapse) one must assume $\gamma < 1$. Although this is strictly unphysical, it may be used for purely illustrative purposes. Note that choosing a non-isothermal stratification leads to a quartic equation in $\tilde{\sigma}$ (ref. 8). (An algebraic error in an earlier analysis for isothermal stratification⁹ resulted in a quartic rather than a cubic equation.)

Inspection of the discriminant of equation (1) shows that increase of ϵ (the ratio of the dynamical to the radiative timescale) leads to a decrease of the growth rate for a tube which is convectively unstable at $\epsilon = 0$. This decrease continues until $\tilde{\sigma}$ acquires an imaginary part, when the discriminant vanishes. It can also be seen, by examining the terms of leading power in ϵ (for $\epsilon > 1$) in the expression for the real part of $\tilde{\sigma}$, that the growth rate tends asymptotically to a constant value.

What are the implications of the above results for solar flux tubes? A straightforward inference is the inhibition by radiation of convective collapse of the tubes. Increase of ϵ implies decrease of r_0 for a given diffusivity χ . Thus, thinner tubes will

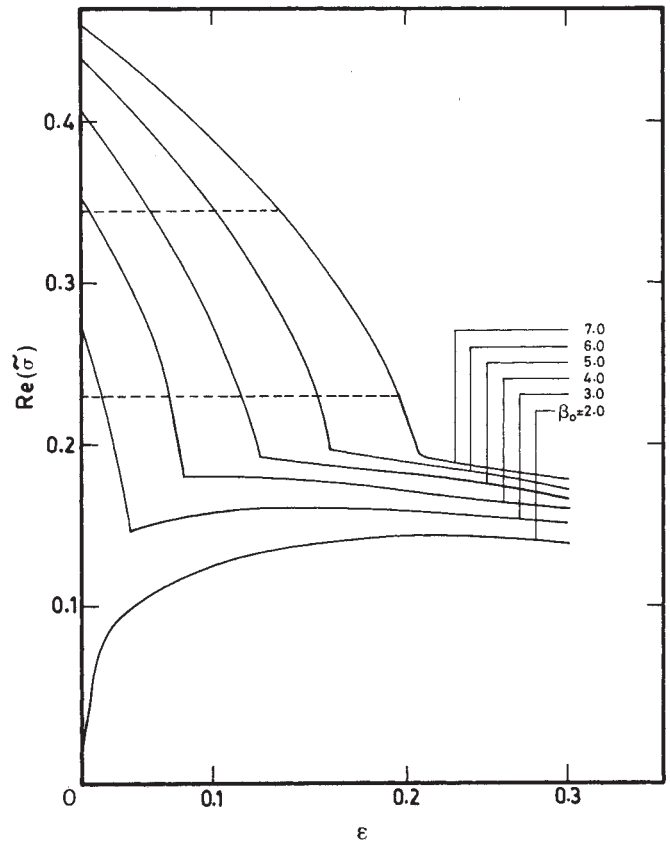


Fig. 1 Plot of $\text{Re}(\tilde{\sigma})$ against ϵ for different values of β_0 . Dashed lines at $\text{Re}(\tilde{\sigma}) = 0.345$ and 0.230 correspond to flux-tube lifetimes of 10 and 15 min, respectively.

collapse to a lesser extent than thicker ones. We shall now examine this phenomenon in a more quantitative manner, without forgetting the simplifications inherent in the assumption of isothermal stratification. We choose Λ_0 corresponding to 10^4 K (representing the region of largest convective instability due to hydrogen ionization), $\gamma = 0.7$ (to yield a very high superadiabaticity of ~ 0.4) and $d = 1,200$ km, assuring a stable tube at $\beta_0 \approx 2.0$ for $\epsilon = 0$. With this choice of parameters, Fig. 1 shows the real part of $\tilde{\sigma}$ as a function of ϵ for different values of β_0 . We assign a value $\beta_0 = 7.0$ for the equipartition field of 700 G, so that $\beta_0 = 2.0$ corresponds to kilogauss intensity. Notice the curious cusps in the curves, which correspond to the transition from purely growing modes to overstable modes.

In the Sun, the flux tubes are presumably jostled around, with frequent rearrangement of the field lines into different concentrations^{10,11}. Thus, a given tube probably exists as a separate identity for a finite lifetime, dictated externally by its environment. Tubes with growth times larger than such externally dictated lifetimes can therefore be considered as 'stable'. In Fig. 1, two horizontal lines (dashed) are drawn at $\text{Re}(\tilde{\sigma}) = 0.345$ and 0.230 . These correspond to stable tubes with lifetimes of 10 and 15 min, respectively.

The 10-min timescale is representative of the normal granulation which rearranges the field lines¹⁰, whereas the 15-min timescale represents the facular granules¹². From the intersection of these horizontal lines with the curves for different values of β_0 (Fig. 1), one can construct a plot of ϵ versus β_0 , showing the demarcation of stable and unstable regimes in ϵ - β_0 space. By choosing $\chi = 10^{10} \text{ cm}^2 \text{ s}^{-1}$ (consistent with the solar value at 10^4 K)¹³ and assuming magnetic flux conservation as well as depth-independent β_0 for the tube, one can write the photospheric radius of the tube as $r_{\text{ph}} = (p_{\text{ion}}/p_{\text{ph}})^{1/4} \cdot (4\tau_D/\epsilon)^{1/2} \text{ km}$, where $p_{\text{ion}}/p_{\text{ph}}$ is the ratio of the pressure at 10^4 K to photo-

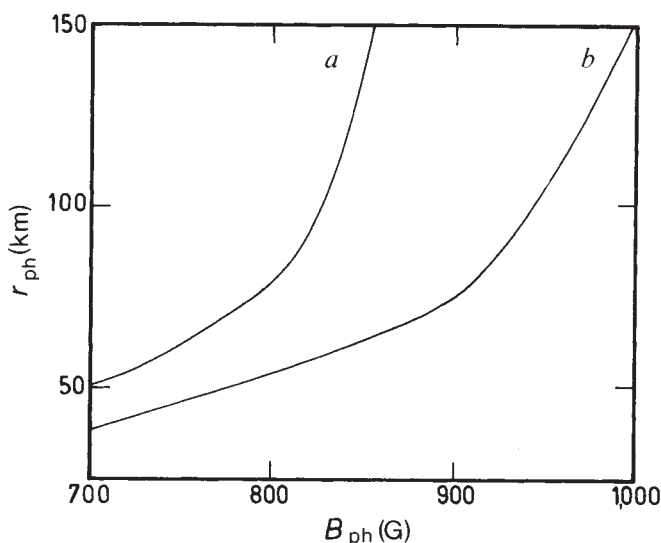


Fig. 2 Predicted photospheric cross-sectional radius of magnetic flux tubes as a function of their field strength, for stable tubes of lifetime 10 (a) and 15 (b) min.

spheric pressure and the dynamical timescale τ_D is expressed in seconds. Similarly, the surface magnetic field can be written as $B_{ph} = 700\sqrt{8/(1+\beta_0)} G$, remembering our assignment of $\beta_0 = 7.0$ to a 700-G tube. Figure 2 shows the resulting plot of r_{ph} versus B_{ph} for the two values of lifetime.

We conclude that stable tubes on the Sun would show a typical distribution of field strength versus size (Fig. 2). At one end of the spectrum, there would be intense kilogauss tubes with small dispersion in strength but large dispersion in size. At the other extreme of the distribution, there would be a group of thin tubes of fairly constant size but with a continuous distribution of field strengths ranging from kilogauss levels down to equipartition values of 700 G. The tubes observed until now from ground-based telescopes¹⁴ have perhaps belonged only to the stronger variety; observations from the new space-based telescopes should reveal the existence of the tubes at the thin end of the spectrum. A further prediction which can be made from Fig. 2 is that longer-lived tubes of a given size are magnetically more intense.

We emphasize the simple isothermal nature of the model which led to the above predictions: for a more precise prediction of the size distribution, the flux-tube model must be improved. A stability analysis¹⁵ of such an improved model has shown a similar influence of the radiative diffusion on the convective collapse, although the instability was not examined in the ϵ - β_0 plane as we have done here. Finally, it must be remembered that mechanisms other than convective interaction might create strong tubes below the photosphere, which could emerge above the surface by buoyancy. In this case, the curves in Fig. 2 should be taken as the stable limit of the weakest field for a given size, or conversely the limit of the largest tube of a given strength. Thus, curves like these can be used to distinguish between tubes concentrated by convection and those formed otherwise.

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Lake acidification and the land-use hypothesis: a mid-post-glacial analogue

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It has been suggested that the recent acidification of lakes owes more to catchment soil acidification than to acid deposition. This can be tested by assessing the effect of catchment soil changes on lake acidity during earlier periods of post-glacial time, when acid deposition can be assumed to have been minimal. In the United Kingdom the mid-post-glacial formation of blanket mires in the catchments of lakes sensitive to acidification provides a suitable analogue. Here we use the results of a pollen and diatom study of a sediment core from a recently acidified lake in Galloway, south-west Scotland³, to show that the lake was acid (pH 5.5-6.0) before peat formation and that no acidification occurred while peat was developing in the catchment. Acidification to pH values <5 occurs only after AD 1850, during the period of increasing acid emissions from fossil fuel combustion. We conclude that soil acidification is not the likely cause of the very low pH values found in many acidified lakes today.

Many lakes in Europe and North America have been acidified in recent decades³⁻⁸, however, arguments persist over the possible causes of acidification. Rosenquist¹, supported by Krug and Frink², has argued that the increase in the quantity of raw humus in lake catchments as a result of land-use change is of primary importance, and Pennington⁹ has attempted to illustrate this process by reference to the post-glacial development of acid lakes in the English Lake District. On the other hand, Seip¹⁰ has explained how sulphate anions from acid precipitation can carry H^+ from soils with low acid-neutralizing capacity into surface waters, and Battarbee *et al.*¹¹ have used palaeolimnological techniques to show that an increase in acid deposition is the only plausible hypothesis to explain the acidification of Galloway lakes with non-afforested catchments, partly by showing that a land-use change as postulated by Rosenquist has not occurred in this region.

Although there is theoretical support and empirical evidence for both mechanisms of surface water acidification, we would like to know which is more important in the recent acidification of lakes in areas where both processes have been occurring². Palaeoecological techniques can be used to separate these factors by considering the influence of catchment changes on a lake at a time when sulphur emissions from industrial sources can be assumed to have been minimal. Such time controls are superior to spatial controls because they allow us to study lakes in areas of high acid deposition, which have already been shown to be recently acidified. Moreover, many important variables such as lake morphometry and the ratio of catchment to lake area can be held constant. Suitable pre-industrial sites and time periods can be identified by pollen analysis and ¹⁴C dating.

In the United Kingdom the mid-post-glacial expansion of blanket mire vegetation in upland areas provides the ideal test situation. The replacement of forest vegetation by *Calluna* heathland and the leaching and paludification of mineral soils to create acid peatland environments has been well established

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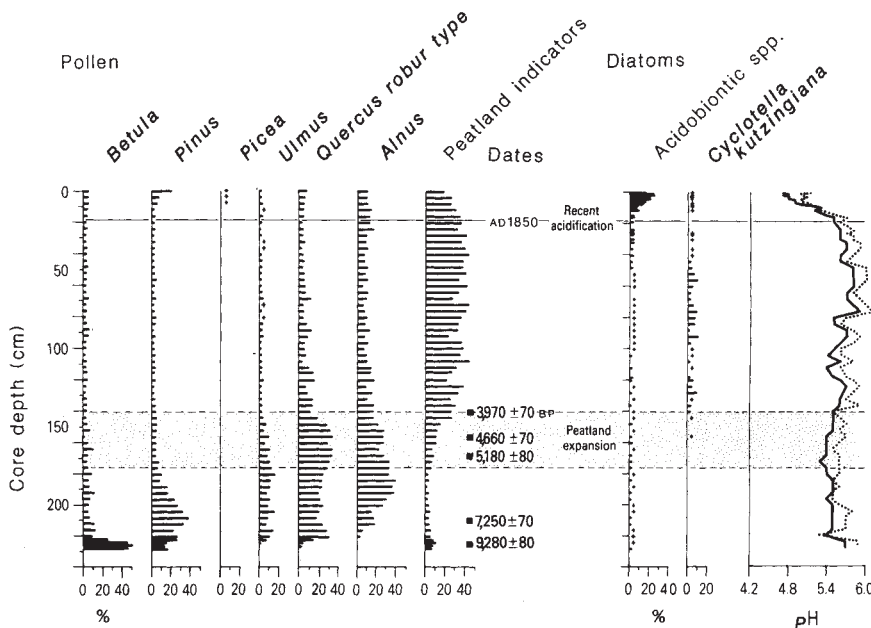


Fig. 1 Composite pollen and diatom diagram for a core from the Round Loch of Glenhead, including ^{210}Pb and ^{14}C dates and pH reconstruction based on Index B (refs 5, 19) and multiple regression of pH preference groups¹⁹. Peatland indicators are *Calluna vulgaris*, *Sphagnum*, *Cyperaceae*, *Succisa* and *Potentilla*; acidobiontic spp. are *Tabellaria quadrisepitata*, *T. binalis* and *Navicula hofleri*. Solid line, Index B (Galloway); dotted line, multiple regression.

by palaeoecologists^{12,13}. Blanket mires of this kind dominate the catchments of lakes in Galloway which have been strongly acidified since 1850³. For one of these sites, Round Loch of Glenhead, we have carried out pollen analysis and ^{14}C dating of a complete post-glacial sediment core to identify and date the development of blanket mire in the area, and we have used diatom analysis to reconstruct the pH history of the loch before, during and after the catchment change.

Pollen data from Round Loch (Fig. 1) show a complete early post-glacial forest succession from *Betula*-*Pinus* (9,280 ± 80 yr BP at 223–227 cm, Fig. 1) to *Pinus*-*Ulmus*-*Quercus*, and then to *Quercus*-*Ulmus*-*Alnus* woodland (7,250 ± 70 yr BP at 208–213 cm, Fig. 1). The woodland was subsequently replaced by an acid peatland dominated by *Calluna*, *Cyperaceae* and *Sphagnum* between 6,000 and 4,000 yr BP. Following this, few changes occurred until the recent afforestation in the region in the past 30 yr, indicated by the increased *Pinus* and *Picea* values in the uppermost sediment (Fig. 1). A detailed pollen stratigraphic examination of the catchment peatland (V.J.J., unpublished data) confirms that the pollen changes in the lake core reflect changes in the local region, and ^{14}C dates for the inception of peat formation in the catchment indicate that acid organic soils were gradually replacing mineral soils and covering exposed bedrock from 9,000 yr BP onwards.

In spite of the early development of acid soils, the diatom record (Fig. 1) shows that neither this nor the later expansion of blanket peats caused an acidification of the lake. On the contrary, at the time of blanket mire expansion there was an increase in *Cyclotella kutzingiana*, a planktonic species that is rarely found in any abundance below pH ~5.5, and an increase in *Fragilaria virescens*, a species with circum-neutral pH preference. These changes indicate a small but significant increase in mean water pH, reflected in the pH reconstruction (Fig. 1), and suggest that leaching of sub-soil mineral horizons by more acidic soil water was responsible for an increase in alkalinity of the lake water. In general, the data show that Round Loch had a pH between ~5.5 and 6.0 throughout the post-glacial period, until the recent post-1850 acidification, when pH fell by almost one unit to the present value of ~4.7.

Previous studies of long-term lake acidification in the United Kingdom^{14–18} appear to be in conflict with these findings, as they all show an acidification trend during the early millennia of the post-glacial period. However, in none of these cases was

the early post-glacial diatom flora as acid as at the Round Loch, probably because none was situated on granitic bedrock, and in all cases the post-glacial acidification trends did not result in a diatom flora characteristic of acid lakes (that is, with negative alkalinity). These data are therefore consistent in showing that nowhere did soil acidification and blanket mire formation during the post-glacial period result in the lowering of lake water pH below ~5.0–5.5.

Soil acidification does not necessarily lead to surface-water acidification, especially where surface waters have low alkalinity and where acids generated in organic horizons can be neutralized by cation exchange and weathering in mineral horizons. Results from the studies cited above^{14–18} show that acidification of lakes from alkaline to slightly acid conditions has occurred during the early post-glacial period, probably as a result of catchment soil changes. The Round Loch data, however, indicate that soil acidification is not a sufficiently effective mechanism to explain the very high levels of acidity found in contemporary acidified lakes. The hypothesis can be rejected in Galloway and must be open to serious question in other regions.

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Non-axisymmetric behaviour of Olduvai and Jaramillo polarity transitions recorded in north-central Pacific deep-sea sediments

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The causes of the reversals of the Earth's magnetic field can be investigated by studying the palaeomagnetic record preserved in rocks. A proper documentation of reversal phenomena should yield valuable information about the generation of the field. Deep-sea sediments provide a globally and temporally distributed set of polarity transition records. Our studies²⁻⁴ of the Olduvai (1.88–1.72 Myr)¹ and Jaramillo (0.94–0.88 Myr)¹ subchrons using deep-sea sediments showed that the time-averaged behaviour of the geomagnetic field during these reversals proved to be reliably recorded, independent of the latitude and longitude of the sample site. Here we test some phenomenological transition models and present directional data, virtual geomagnetic poles (VGPs) and related directions in rotated declination, inclination (D' , I') space⁵, using two records of the Olduvai reverse–normal–reverse (R–N–R) and two records of the Jaramillo R–N–R polarity transitions. These data are better explained by the flooding rather than the standing field model, but they also indicate a degree of asymmetry of the transitional field.

The palaeomagnetic data presented here are associated with a pair of sequential reversals (R–N and N–R) from a fully azimuthally-oriented piston core^{2,6,7} (K78030; 18°55.9' N, 160°70.8' W) taken near the Hawaiian island of Kauai. The other two records are from two other fully azimuthally-oriented piston cores^{2,6,7}: K76113 from low latitude (2°40.1' N, 178°45.1' W) and K7501 from a mid-latitude site (37°22.4' N, 179°36.1' W), which encompass the Olduvai reversals (R–N, K76113 and N–R, K7501).

The transitions studied were sampled using a 'high-resolution' technique²⁻⁴. The measurements derived from each sample represent a signal averaged from as little as 200 to as much as 1,000 years of post-depositional remanent magnetization (PDRM) history (considering the sediment accumulation rates of the cores studied). Thus, with a typical 3-mm offset between boxes, field fluctuations of periods between 200 and 600 years should, at least theoretically, be within the reach of our high-resolution technique. The four records discussed here are characterized by high palaeomagnetic stability of the samples within and outside the transition zones²⁻⁴.

Figures 1 and 2 show the highly detailed VGP paths associated with the two sets of transitions. The VGPs are plotted with respect to their site longitude⁵ and are those which represent intermediate directions, as well as some outside the transition zones. The pattern of change in field directions is clear for the two transitional sets (Olduvai and Jaramillo subchrons) and is sufficient to apply Hoffman's⁸ test (to distinguish between the flooding model and the existence of a stationary (non-reversing) portion of the field during axial dipole decay and regeneration, using palaeomagnetic data corresponding to back-to-back (R–N and N–R) polarity transitions). During the onset of the Olduvai subchron R–N transition (core K76113), the VGPs representing the field remain southerly while migrating to nearly equatorial latitudes. The ancient field apparently underwent a rapid set of 'flips' during the transition, like those observed in several other detailed records^{9,10}. From the southerly latitudes, the VGPs migrate up to the Northern Hemisphere to the expected normal field direction. The path is clearly near-sided and is longi-

tudinally confined. In the N–R reversal (core K7501) at the termination of the Olduvai subchron, a different sequence is observed. The termination path is more complicated than the onset path and is characterized by more intermediate VGPs (fewer in the Southern Hemisphere with respect to the VGPs in the Northern Hemisphere) and bigger loops in its traverse from northern latitudes to southern latitudes. These loops may show rapid fluctuations of the transitional field. The path can be classified as a far-sided path even though some of the VGPs lie in the near-sided section of the plot ($\pm 90^\circ$ from the site longitude). This path also exhibits a longitudinal confinement, as does the onset transitional path. Thus, both reversals are characterized by transitional VGP paths which lie along meridional bands on either side of the 90° meridian, but the paths are not completely antipodal.

Analysis of the two Jaramillo transitional paths recorded by core K78030 (Fig. 2) shows that the two paths are dissimilar even though they represent records of a back-to-back transition zone from the same site. For instance, the R–N records display intermediate VGPs lingering at low southerly latitudes in the vicinity of the 90° meridian, with a sudden transit to the Northern Hemisphere where big loops occur before the final move to the expected normal direction. The path could be classified as near-sided because most of the intermediate VGPs reside within $\pm 90^\circ$ of the site longitude. The N–R Jaramillo transition path is more complicated than the R–N Jaramillo path. The VGPs in the Northern Hemisphere linger briefly before their transit to southern latitudes where they seem to migrate, forming loops in the Southern Hemisphere before their final move to the expected reversed field direction. This path is hard to classify as near-sided or far-sided because the VGPs lie on both sides of the meridian 90° from the site longitude. Thus, the transitional field of these two reversals was different. A characteristic of both the Olduvai sets and the Jaramillo sets is that the paths are not antipodal. A comparison of all four paths—between sites and within sites—shows that they are characterized by individual features peculiar to each transition; and overall, the data indicate that the reversal process is asymmetric, such that each reversal sense is associated with a different field configuration.

Figure 1 shows the VGP data, as well as the transition data rotated using Hoffman's method⁵. The polar (D' , I') stereographic plot clearly shows that at the onset of the Olduvai subchron (K76113) the field displays an initial near-sided vector movement in quadrant 'n' (the first 10 rotated directions which are apparently closer to the reversed polarity and which represent the initiation of the reversal—still not part of the transitional field); then, a swing to the other quadrant 'n'; and finally a movement to normal polarity in the 'N' region of the diagram. These last two steps are part of the strictly transitional field behaviour. Thus, from this record alone, it can be concluded that the field geometry during the onset of the Olduvai reversal is consistent, but not uniquely so, with a hypothesized transi-

Table 1 Results of rotated intermediate directions and VGPs of Olduvai and Jaramillo polarity transitions

Transition	Sense of transition	VGP	(D' , I')
Jaramillo			
Termination K78030	N–R	f	f
Onset K78030	R–N	n–f	N
Olduvai			
Termination K7501	N–R	f	f
Onset K76113	R–N	n–N	n–N

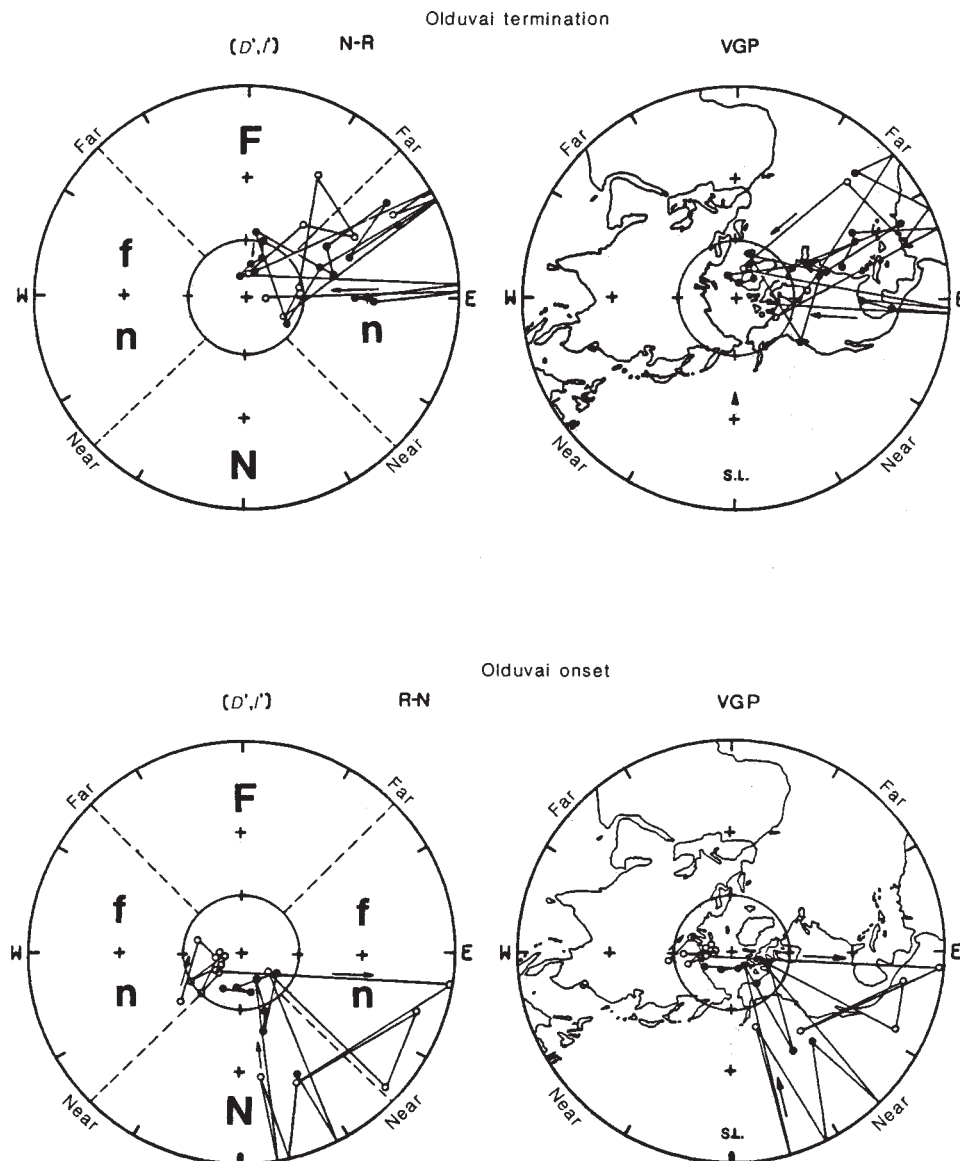


Fig. 1 Polar (D' , I') stereographic display showing regions possessing different degrees of near-sidedness and far-sidedness. Rotated directional path (left; $\circ$, upper hemisphere; $\bullet$, lower hemisphere) and VGP path (right; $\circ$, Southern Hemisphere; $\bullet$, Northern Hemisphere) associated with the onset (core K76113) and termination (core K7501) polarity transitions of the Olduvai subchron. Circles have been drawn at $|I'| = 60^\circ$ and $|VGP \text{ latitude}| = 60^\circ$. The site location is also indicated on the VGP plot along the site longitude (SL).

tional palaeofield geometry which is significantly zonal (rotated directions in the 'N' region of the (D' , I') diagram), as well as a non-axisymmetric palaeofield geometry (rotated transitional directions in the 'n' region). Figure 1 also shows the transitional characteristics of the termination of the Olduvai subchron (core K7501). The record displaying the rotated (D' , I') directions shows that the path lies on the far-side region of the polar diagrams. Initially, the reversal is controlled by a consistent axisymmetry, and during the middle and final stage of the reversal the field was apparently dominated by non-axisymmetric terms; most of the rotated directions are located in the 'f' region.

The two transitional records (Olduvai reversals) present entirely dissimilar characteristics. For instance, the rotated directions of the R-N reversal record are near-sided, whereas the rotated directions of the N-R record are far-sided. The two records do not present antipodal directions; instead, at different stages of the reversals some of the directions are consistent with a dominating axisymmetry and others with non-axisymmetric terms.

The rotated directions and VGP paths for the two back-to-back reversal records of the Jaramillo subchron (core K78030, from ~300 km from Kauai) are shown in Fig. 2. The (D' , I') rotated directions of the R-N reversal record of the Jaramillo subchron display almost a total dominance of a consistent axisymmetry.

Such rotated directions are located in the 'N' region of the polar diagram. This reversal record shows that at the initial stage of the transition some of the directions are located on the far side of the polar diagram, and at some stage two directions are in the 'n' quadrant. Nevertheless, the path could be classified as near-sided. Figure 2 also shows the N-R reversal path of the Jaramillo subchron. This is a unique path with respect to the other three paths presented here: it is more complex than the R-N Jaramillo path and displays rotated directions on both the near and far side of the polar diagram. One group of rotated directions is in close proximity to the $D' = 90^\circ$ great circle (that is, the plane containing the horizontal east-west line and the axial dipole field direction). Other groups of directions are along the $D' = 180^\circ$ great circle (the plane containing the north-south line and the axial dipole field direction). In general, this transitional record is characterized by stages of the reversal that are in the 'N', 'n' and 'f' regions of the polar projection plot, although the path lingers mostly in the far-sided region of the diagram.

Each of the records presents individual characteristics, perhaps indicating that the harmonic content of each is different, even in the case of the two records of the Jaramillo subchron taken from the same core³. From a study of two older (Pliocene) Kauai back-to-back reversal records, Bogue and Coe concluded that those two paths are distinctly near-sided¹⁰⁻¹². Our nearby

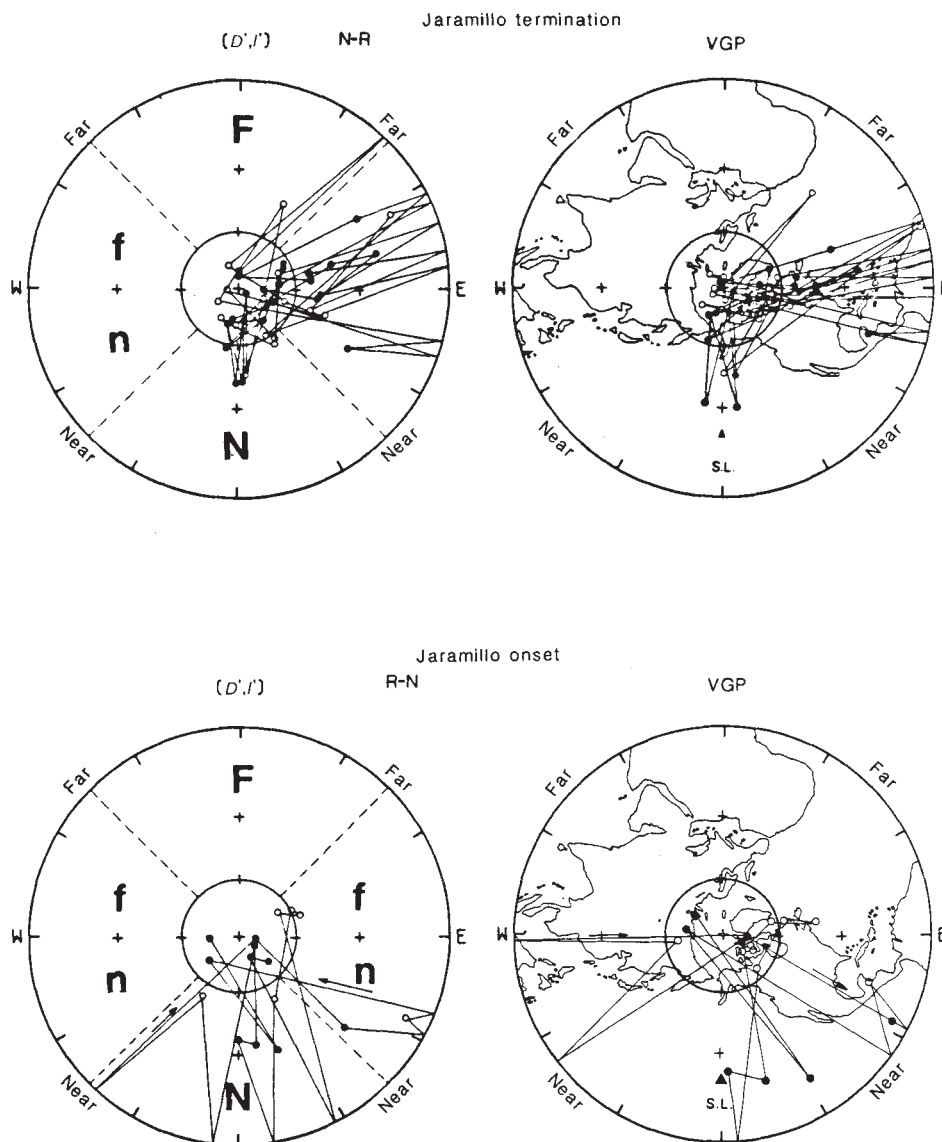


Fig. 2 Polar (D' , I') stereographic display showing regions possessing different degrees of near-sidedness and far-sidedness. Rotated directional path (left) and VGP path (right) associated with the onset and termination polarity transitions of the Jaramillo subchron (core K78030). Circles have been drawn at $|I'| = 60^\circ$ and $|VGP \text{ latitude}| = 60^\circ$. The site location is also indicated on the VGP plot along the site longitude (SL). Symbols as in Fig. 1.

core (K78030) that recorded the Jaramillo back-to-back reversals indicate that the R-N record is near-sided, whereas the N-R record is classified as a far-sided path using Hoffman's⁸ terminology. However, certain common characteristics must be taken into consideration. One is the site dependence of the transitions: the two R-N reversal records are near-sided and the two N-R reversal records are far-sided, or at least most of the rotated directions are on the far side of the diagram (for example, Olduvai termination). All four transition records show intermediate rotated directions residing in regions 'n' and 'f' that are only marginally near- and far-sided respectively (see Table 1). All four records are unambiguously associated with significantly non-axisymmetric transitional fields. Another common feature of these paths is that all tend to reside on the American continent. As shown in Figs 1 and 2, the VGP paths are clustered about the 90° meridian. This area is also where the VGP paths from Crete are located¹³.

Which of the current models for transitional field behaviour explains the characteristics of the Olduvai and Jaramillo records discussed here, the standing field model¹⁴⁻¹⁶ or the flooding model^{17,18}? The former predicts identical transitional field behaviour for repeated reversals as observed from a single site, and requires the postulated standing field to persist for several million years, whereas the latter treats the Earth's fluid core as a distributed source of magnetic field in which polarity transi-

tions begin with a localized reversal of field sign in a small region of the core, an effect that consequently spreads or floods through the rest of the source region. If the reversal starts at either of the poles or at the equatorial zone of the core, basically axial quadrupolar, or axial octupolar fields, respectively, will predominate during the intermediate stages of the reversal process. For the purely axisymmetric, and consequently the simplest cases, the flooding will begin at a pole (a predominantly quadrupolar transitional field) or along the equatorial band of the fluid core (an octupolar field at the midpoint of the reversal). Depending on the sense of the reversal and the hemisphere of the observer, the VGP path will be near-sided (same longitude as the site) or far-sided (180° away from the site). This is the basis of Hoffman and Fuller's method¹⁹ for determining the origin point of flooding, which involves the comparison of palaeomagnetic records of both R-N and N-R reversals.

The standing field model cannot successfully explain the diverging behaviour of the paths because it predicts identical transitional field behaviour for repeated reversals when viewed from a single site. Moreover, Bogue and Coe¹¹ have shown that the palaeointensity records of the Kauai transitions are not consistent with the standing field model. The same conclusion was reached by a palaeointensity study of the Steens Mountain polarity transition²⁰.

The generalized flooding model can successfully explain these data. For the two successive reversals (Jaramillo subchron), the model predicts (so long as a reversal is initiated at the same zone in the core each time) transitional VGP paths that differ in longitude by 180° . In general, for core K78030 the two records are not antipodal (R-N near-sided and N-R far-sided), and the same conclusion can be drawn from the Olduvai subchron paths, even though the two records are from two different localities. The salient directional features shared by all of these records are the overall longitudinal confinement of most intermediate VGPs and respective rotated directions well to the east of the sites; and the typical location of the intermediate VGPs over the Americas, the southeastern Pacific and the western Atlantic. None shows smooth pole-to-pole paths; all define major loops (including east-west swings, for example, in the Olduvai onset record, Fig. 2) before settling into the expected opposite polar positions. The important issue here is that the VGP paths are not antipodal. The rotated directions of all these data prove that the reversal process is asymmetric, such that each transition sense is associated with a different field configuration. Indications that the flooding process is itself asymmetric have been recently reported from the studies of the Kauai and Steens Mountain transitions^{11,20}.

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Late Palaeozoic to early Mesozoic evolution of Pangaea

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Several possible configurations of the Pangaea supercontinent have been suggested for the interval from late Carboniferous to early Triassic. Here we re-examine the palaeomagnetic basis for these models, emphasizing the trends of the paths of apparent polar wander for the individual components of the supercontinent rather than simply averaging poles of presumed similar age. Two of the alternatives, Pangaea B and C, may result from averaging poles of dissimilar age along common polar wander paths, giving rise to spurious tectonic displacements. The most likely model appears to be the formation, in late Devonian time, of a modified Pangaea (A2) followed by evolution to the traditional configuration (A) during the Triassic.

Hallam¹ reviewed geological information concerning the possible configuration of the Pangaea supercontinent in Permo-Carboniferous time and concluded that the evidence favours an unchanged configuration from the late Carboniferous to the Jurassic. However, the palaeomagnetic data disagree with such a model. In particular, late Palaeozoic poles from Gondwanaland are distinct from those obtained for the northern continents when plotted on the 'traditional' Pangaea reconstruction. Solutions to this problem range from an anticlockwise rotation of Laurasia relative to Pangaea of $\sim 20^\circ$ about a pole in the western Sahara²⁻⁵, to a rather larger rotation of $\sim 35^\circ$ about a different pole⁶⁻¹¹, to a drastic model in which northwestern South American is juxtaposed with southern Europe¹². These solutions have been designated Pangaea-A2, Pangaea-B and Pangaea-C respectively^{1,10}. Figure 1 shows the alternative reconstructions for the Permian, plotted on the same scale and in palaeomagnetic coordinates.

Most of the contrasts between the competing models are due to differences in palaeolongitude of the component fragments;

thus, palaeomagnetic data from a restricted interval of geological time cannot easily distinguish between these alternatives. However, a careful examination of the apparent polar wander paths of the component continental blocks for a longer period of time may allow some of the models to be discounted. This is the approach we have followed in our re-evaluation of the palaeomagnetic data.

Carefully selected palaeomagnetic poles from the interval 180-320 Myr (ref. 13) have been returned to their palaeogeographical positions in the alternative reconstructions. The data have then been sampled in 20-Myr non-overlapping windows and Fisher statistics calculated for the various continental fragments in an attempt to examine the implications of the various models.

The poles were selected from recent lists of data thought to represent the ancient geomagnetic field direction, such as those given by Embleton¹⁴ for Australia and East Antarctica, by Van der Voo¹⁵ for North America, and by Brock¹⁶ for Africa and Madagascar. In addition, more recent results (to 1983) have been incorporated. Results for which the time of magnetization could not be estimated to within 25 Myr were excluded. All the poles used conform to the minimum reliability criteria of McElhinny¹⁷ and are based on studies in which AF (alternating-field) and/or thermal cleaning techniques were employed. Results from within or east of the Urals have not been included because of the likelihood of relative motions between Europe and Siberia during at least part of the period of interest. Stratigraphic ages have been referred to the timescale of Harland *et al.*¹⁸ and K-Ar dates have been standardized to the decay constants given by Steiger and Jaeger¹⁹. The assigned ages, together with the absence of Russian data and the inclusion of some more recent studies, constitute the chief differences between this dataset and that used by Morel and Irving¹⁰.

Any time window for data grouping must be narrow enough to preserve the main features of apparent polar wander (APW) paths, yet broad enough to allow sufficient poles to be included to define precise means. A width of 20 Myr was chosen as the minimum presently permitted by the number and quality of available data. Having returned the poles to the various reconstructions, Fisher statistics were calculated in Africa-fixed coordinates for each continent within each window, assuming a geocentric axial dipole field. The means and their associated A_{95} standard error circles are given in Table 1 for the four

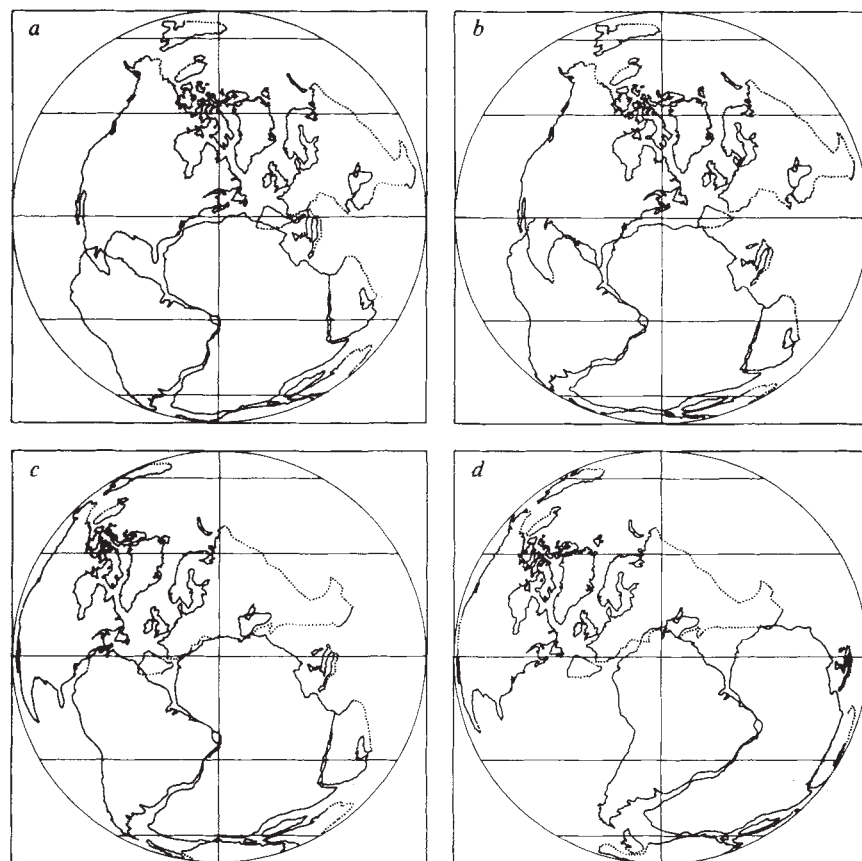


Fig. 1 Alternative models of the Pangaea supercontinent, orientated using early Permian (270 Myr) palaeomagnetic data. Orthographic projection. *a*, Pangaea-A, constructed using published sources²⁶⁻³¹; this is comparable to the classic reconstructions of Wegener³² and Bullard *et al.*³³. *b*, Pangaea-A2 reconstruction, obtained by applying an additional rotation of 20° about a pole in the north-west Sahara to Laurentia-Baltica, after ref. 2. *c*, Pangaea-B reconstruction, obtained using the rotations of Irving¹¹ for Laurentia, Baltica and Gondwana. *d*, Pangaea-C of Smith *et al.*¹², showing a large Tethyan offset of Gondwana and the northern continents of several thousand kilometres.

Table 1 Mean poles of continental fragments with respect to the different Pangaea reconstructions, with Africa fixed in present-day coordinates

Mean pole										
Age (Myr)	Pangaea-A		Pangaea-A2		Pangaea-B		Pangaea-C		A_{63} (deg)	N^*
	Lat. (deg)	Long. (deg)	Lat. (deg)	Long. (deg)	Lat. (deg)	Long. (deg)	Lat. (deg)	Long. (deg)		
Europe										
210	61.5	239.0	44.2	253.3						1
230	54.9	227.3	39.0	243.4						1
250	50.8	216.2	36.8	233.7	47.9	248.6	38.2	263.0	1.9	5
270	37.3	226.6	22.0	238.2	33.9	240.1	32.2	245.6	1.8	19
290	34.3	224.5	19.3	235.9	33.0	236.1	33.7	241.9	3.0	8
310	33.9	220.0	19.8	231.9	35.5	232.6	37.4	241.3	4.8	7
North America										
190	66.8	242.4	49.1	256.7					2.9	6
210	70.0	233.0	53.0	252.4					4.7	4
230	62.2	215.9	47.7	238.4					6.2	4
250	55.9	222.1	40.7	239.9	45.6	254.4	31.3	266.4	2.9	8
270	46.3	218.0	32.1	233.6	42.5	241.4	35.3	255.4	1.5	11
290	36.6	225.7	21.4	237.3	31.9	236.7	29.8	243.8	2.8	3
310	37.1	221.1	22.7	233.6	34.9	234.2	33.5	244.3	2.9	3
Africa										
190	70.1	246.6							3.9	7
210	67.2	246.1							6.4	5
230	67.3	250.9							4.3	6
250	60.6	261.0								2
270	31.7	241.8							5.3	3
290	29.0	240.0								1
310	16.1	242.5								1
South America										
190	50.8	242.2								2
210	68.2	228.2								1
230	58.4	247.3							3.4	5
250	50.1	252.4							4.2	5
270	46.5	243.2								2
290	23.2	237.9								2
310	23.3	230.5							4.4	4

* N is the number of poles averaged to obtain the mean pole for an interval.

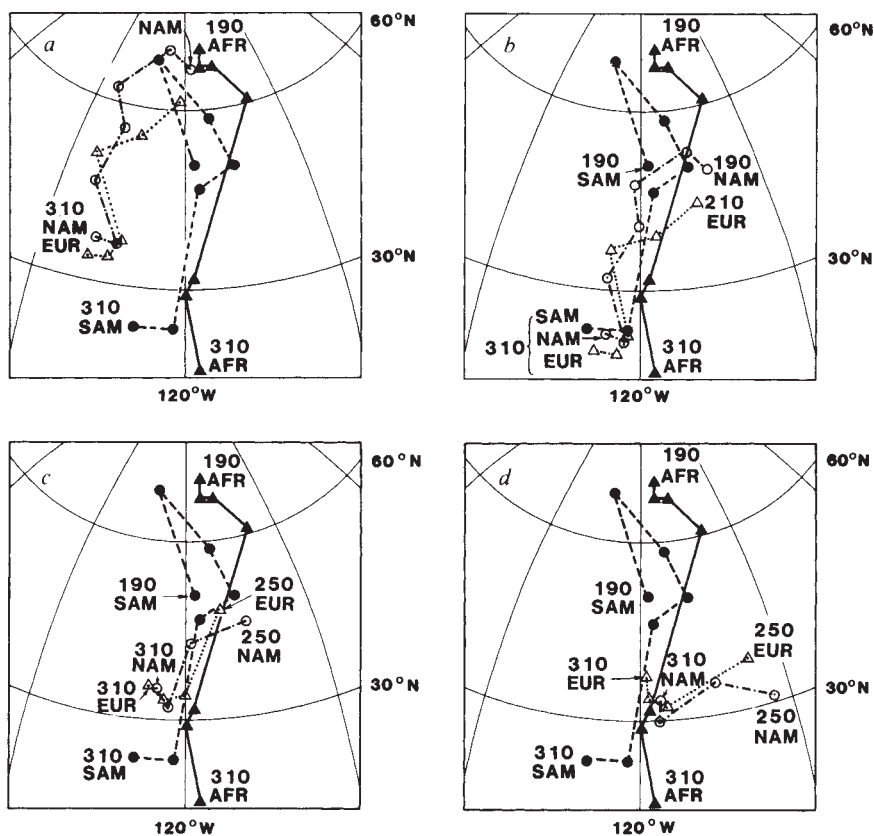
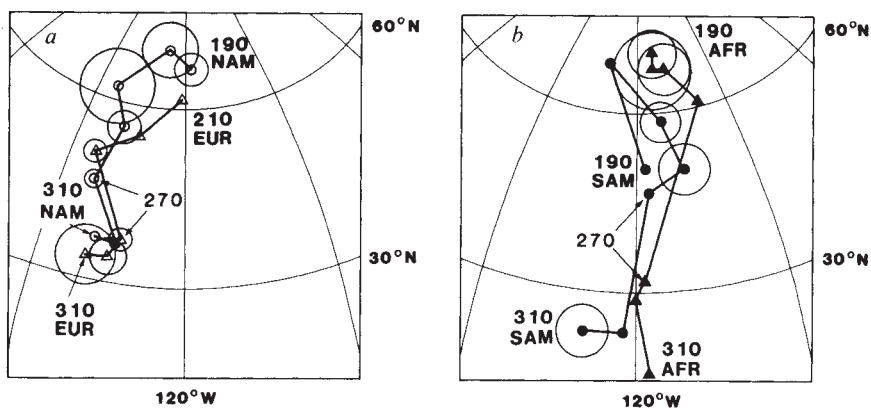


Fig. 2 Positions of mean palaeopoles and standard error (A_{63}) circles relative to an Africa-fixed Pangaea-A reconstruction, showing the general agreement of trends. However, detailed examination reveals differences in timing; for example, the 270-Myr means for Europe and North America disagree, as do those for South America and Africa. Lambert Equal Area projection. *a*, North America (○) and Europe (△). *b*, South America (●) and Africa (▲).

Fig. 3 *a*, APW paths of the northern (North American, European) and southern (African, South American) continents plotted relative to Pangaea-A in Africa-fixed coordinates. Symbols as for Fig. 2. Equal area projection. *b*, As for *a* but plotted relative to Pangaea-A2. The discrepancy between northern and southern continents before 210 Myr is now reduced, although the timing of motion along the common path varies between continents. *c*, As for *a*, but plotted relative to Pangaea-B for the interval 250–310 Myr. *d*, As for *c*, but plotted relative to Pangaea-C.



models. Note that no results are given for times younger than 250 Myr for either B or C, as these models were only intended for Permo-Carboniferous time.

Figure 2 illustrates the poles calculated with respect to Pangaea-A. Figure 2*a* shows that the trends of the APW paths for Europe and North America, especially for pre-Triassic time for which more reliable data are available, are very similar. Apart from the oldest two intervals, however, the A_{63} circles do not overlap and there is a clear offset in age along the common path. On the other hand, there is good agreement between the 290-, 270- and 250-Myr poles from North America and the 270-, 250- and 230-Myr poles for Europe, respectively. This suggests a possible error in intercontinental correlation of up to 20 Myr, or widespread remagnetization of the North America formations after a similar period of time. Similarly, although less obviously, the paths for South America and Africa (Fig. 2*b*) follow broadly similar northward routes, but with quite large divergences for individual windows, for example, 260–280 Myr.

Part of these differences may be attributable to dating errors for individual results, coupled with rapid polar wander. The discrepancies do appear to be systematic, however, and one

suspects that a more fundamental problem exists concerning the accuracy of the stratigraphic correlations on which most of the assigned ages are based. In addition, many poles derive from studies of non-fossiliferous red sediments, for which the mechanism of remanence acquisition remains to be clearly demonstrated.

The observed differences cast considerable doubt on the Pangaea-B and -C reconstructions, because both of these models were constructed by moving Laurasia and Gondwanaland until their Permian and late Carboniferous poles coincided. If there are systematic errors in the correlation of these poles, then these reconstructions may be viewed as artefacts of the dating errors. An example of this may be seen in the Pangaea-B model, which requires a small ocean separating Europe and North America to satisfy an observed difference between their mean palaeopoles¹⁰. If, as suspected, this difference arises from dating errors along a common polar wander path, then no such separation (for which geological evidence is absent) is required.

One conclusion that can be drawn from the foregoing is that comparison of APW path segments is to be preferred to the simple averaging of supposedly contemporaneous poles. Figure

3 shows the polar wander paths for the four main continents (North America, Europe, Africa and South America) plotted with respect to Pangaea-A, A2, B and C in Africa-fixed coordinates. Figure 3a, b clearly illustrates the discrepancy between the paths for the northern and southern continents in the Pangaea-A reconstruction for times before 210 Myr, and the improvement for this time interval obtained if one assumes Pangaea-A2.

In Fig. 3c, d the paths are superimposed for reconstructions B and C, respectively, but only for the period 310–250 Myr, because it is only for this earlier period that these reconstructions have been suggested. With the exception of Pangaea-B for a period of time centred on 270 Myr, there is little similarity between the polar wander paths for the northern and southern continents in either reconstruction. We stop short of suggesting an evolution A2 to B to A2 to A, which would imply implausibly high rates of plate motion, and on the basis of the comparison of APW paths consider it unlikely that Pangaea ever existed in the form of configuration B or C.

It now seems clear from palaeomagnetic, biogeographical and stratigraphic evidence that the configuration of Pangaea immediately before its disruption was similar to that portrayed in Fig. 1a. For earlier times, uncertainty remains. There is doubt about the precise timing of the final collision between Gondwanaland and Euramerica which brought Pangaea into being. Depending on the interpretation of Devonian palaeopoles from Gondwana, this collision may have occurred either during the early Carboniferous or as early as late Devonian²⁰. The exact configuration at these times is not known with certainty, but published reconstructions favour Pangaea-A2^{21,22}.

Several workers^{3,5} have noted the close agreement between the fossil faunas and tectonic structures of southern North America (such as the Oachita and Marathon belts) and those of northern South America (such as the Venezuelan Andes), and have suggested that better alignment of similar provinces can be achieved when the tighter A2 fit is adopted. If an A2 to A transition is accepted, the geological problem is that of locating the structures along which the transition took place, and determining their nature and displacement. A suitable zone of transcurrent motion is most plausibly located along part of the present-day continental margins of eastern North America and north-west Africa. To accommodate such motion in a simple fashion, the Euler pole for A2 would need to be modified, as it does not agree in detail with such a transition zone. This subject will be dealt with elsewhere.

The notable Permo-Triassic volcanism of the Alpine region *sensu lato*, interpreted as representing the break-up of the region, was followed by the subsidence of huge late Triassic and early Jurassic carbonate platforms, whose creation pre-dates the Pangaea^{23,24}. The Alpine cycle began in Triassic or possibly Permian time, but the Atlantic Ocean did not begin to open until the middle Jurassic. The observed tectonic pattern might well reflect mid- to late-Triassic plate margins formed by the A2 to A transition.

In view of this discussion of polar wander paths, and the geological arguments advanced by Hallam¹, both Pangaea-B and Pangaea-C seem less likely candidates, possibly resulting from uncertainties in the dating of components of remanence and/or intercontinental stratigraphical correlation. The most probable evolution appears to be formation of a supercontinent resembling Pangaea-A2 during the late Devonian²¹, followed by Permo-Triassic readjustments involving dextral shear, and the reorganization of fragments in the Gulf of Mexico and Mediterranean regions. This was accompanied by rapid motion of all the major continents with respect to the poles. Siberia and the Asian components of Pangaea (Kazakhstan, China, and so forth) were probably sutured to Europe during Permo-Triassic time¹⁷. By the late Triassic, Pangaea-A was in existence, and, although probably undergoing extension in early Jurassic time, remained intact as a supercontinent until break-up in the middle Jurassic²⁵.

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Mantle heterogeneity beneath the Nazca plate: San Felix and Juan Fernandez islands

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The islands of San Felix, San Ambrosio and the Juan Fernandez group comprise one of the few subaerial occurrences of intraplate oceanic volcanism between the Mid-Atlantic Ridge and the East Pacific Rise south of 20° S, and are therefore valuable in the assessment of large-scale geochemical heterogeneities in the Earth's mantle^{1–3}. We present here the results of a preliminary Sr, Nd and Pb isotopic study of basic lavas from these islands. All samples show a moderate Dupal³ signature (anomalous Sr, Nd and Pb isotopic compositions). Basalts from the Juan Fernandez group lie close to the mantle plane⁴, whereas basalts from San Felix display unusual isotopic characteristics which plot well below the Nd–Sr oceanic array. Based on this data, we propose the delineation of a new mantle array, linear in Nd–Sr–Pb isotopic space, with Tubuaii and Walvis Ridge as end members. The mixing relationships exhibited by this array suggest that both end members are geographically contiguous, and probably reside in delaminated sub-continental lithosphere.

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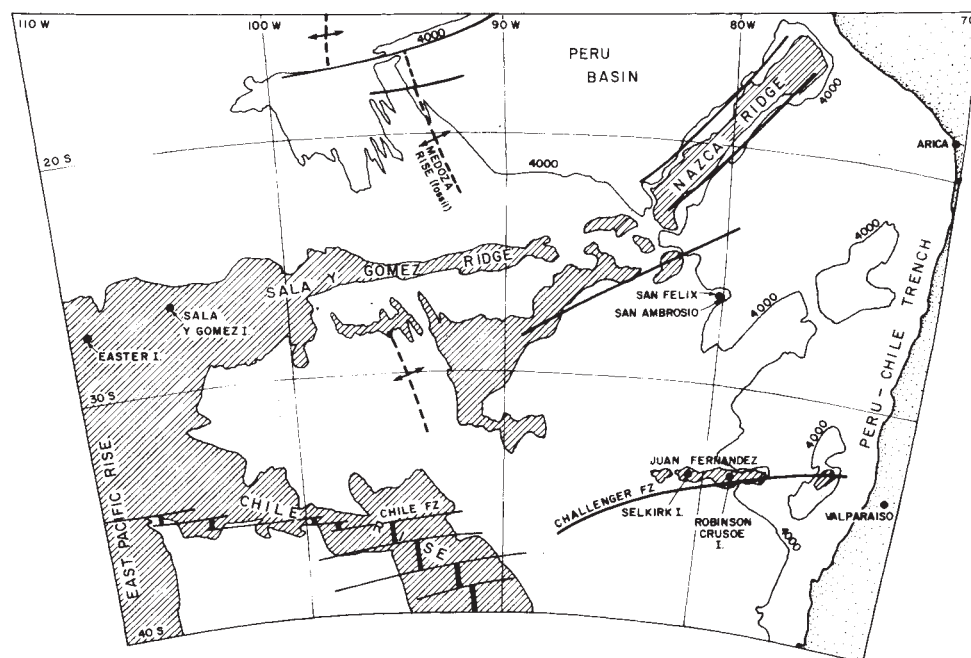


Fig. 1 Generalized tectonic map of the South-East Pacific, showing the location of San Felix, San Ambrosio and Juan Fernandez islands (modified from ref. 42). Shaded areas represent depths less than 3,500 m; 4,000 m contours are also shown. Dashed lines represent extinct spreading ridges. San Felix and San Ambrosio are not related to present or older spreading systems or fracture zones, nor is there any convincing older hot-spot trace extending to the east from these islands. The Juan Fernandez islands lie along an east-west-trending bathymetric ridge, probably associated with the Challenger fracture zone; this ridge may represent a hot-spot trace, as some bathymetric expression can be traced up to 500 km eastwards. Robinson Crusoe island lies on ~38-Myr-old crust; San Felix and San Ambrosio lie on ~42-Myr-old crust.

The Juan Fernandez islands are located on a N80° E-trending aseismic ridge, the Juan Fernandez Ridge, whereas San Felix and San Ambrosio do not appear to be associated with any significant tectonic or topographic feature (Fig. 1). San Felix and San Ambrosio (26°25' S, 79°59' W) are small (<3–4 km²), and located 20 km apart; although no historic eruptions have been reported, fresh-looking pahoehoe lava flows and fumarole activity on San Felix were observed by Willis and Washington⁵. These islands are mainly platforms bounded by steep cliffs, and consist largely of interbedded lavas and deeply weathered pyroclastics intruded by dikes³.

The Juan Fernandez islands include one large island, Robinson Crusoe (~50 km², at 33°37' S, 78°50' W), a much smaller nearby islet, Santa Clara, and A. Selkirk Island, 200 km to the west. Robinson Crusoe island is heavily eroded, with no historic record of volcanic activity, although evidence of submarine volcanism immediately west of Robinson Crusoe has been reported^{6,7}. An extensive volcanic sequence of >2,200 m thickness is exposed on Robinson Crusoe island⁸. The lowest unit (Punta Larga, >800 m thick) consists of extensive basalt flows with rare interbedded pyroclastics. The middle unit (Puerto

Ingles, ~1,200 m) consists of olivine-phyric to picritic basalts interbedded with a relatively greater proportion (~30%) of pyroclastics. Basalt samples from this unit have been dated by K–Ar at 3.1–3.5 Myr (ref. 9). The youngest unit (Bahia del Padre, ~250 m thick) is made up mainly of pyroclastics, with minor olivine basalt flows.

Samples collected from San Felix in 1923 by Willis⁵ include weathered tuff, trachyte and basanite. Of these, we chose for analysis one fresh basanite and one sample of basanite glass, occurring as small inclusions in a palagonitized basanite tuff (Table 1); a fresh tephritic basalt⁵ from San Ambrosio was also analysed. Basalts from Robinson Crusoe are transitional to alkalic, and vary in freshness and olivine phenocryst content.

The basanites from San Felix and San Ambrosio are similar in major- and trace-element composition (D. Gerlach and S. R. Hart, unpublished data) to basanites from alkalic suites of Atlantic oceanic islands such as Fernando de Noronha, St Helena and Tristan da Cunha (refs 10, 11; D. Gerlach and J. Stormer, unpublished data). The basalts of Robinson Crusoe are similar in their major- and trace-element compositions to transitional and alkalic basalts from several Hawaiian vol-

Table 1 Chemical and isotopic data for volcanics from the Juan Fernandez and San Felix islands

Sample no.*	Lithology†	SiO ₂	K ₂ O	Rb	Sr	Ba	⁸⁷ Sr/ ⁸⁶ Sr	¹⁴³ Nd/ ¹⁴⁴ Nd	²⁰⁶ Pb/ ²⁰⁴ Pb	²⁰⁷ Pb/ ²⁰⁴ Pb	²⁰⁸ Pb/ ²⁰⁴ Pb
1 (30MT0182)	AOB	46.71	0.75	15.3	452	182	0.703714 ± 41	0.512847 ± 16	—	—	—
2 (5MT0283)	TAB	48.27	1.04	20.3	554	244	0.703654 ± 23	—	19.042	15.596	38.872
3 (11MT0182)	AB	47.00	0.75	7.6	430	229	0.703512 ± 27	0.512882 ± 19	19.094	15.595	38.899
4 (28MT0182A)	AOB	45.82	1.11	26.0	629	279	0.703779 ± 25	0.512818 ± 19	19.214	15.627	39.099
5 (59MT82B3)	TAB	47.75	0.74	18.6	483	180	0.703634 ± 30	—	19.129	15.609	38.999
6 (14MT0182)	TAB	48.13	0.50	2.7	461	243	0.703581 ± 25	0.512835 ± 18	19.045	15.597	38.886
7 (10MT0283)	AOB	46.01	0.90	18.6	497	239	0.703762 ± 42	0.512831 ± 37	19.130	15.595	38.958
8 (52MT0282)	B	48.24	0.72	—	—	—	0.703629 ± 25	0.512840 ± 40	—	—	—
9 (55MT0282)	B	47.61	1.48	—	—	—	0.703625 ± 26	0.512855 ± 18	—	—	—
10 (99653)	Bs	45.56	3.27	71.4	1447	891	0.704120 ± 27	0.512585 ± 19	18.960	15.569	38.871
11 (99656)	Bs	43.48	3.06	66.3	1359	815	0.704122 ± 18	0.512552 ± 16	19.312	15.602	39.329
12 (99657)	Te	45.88	2.32	51.8	1218	689	0.703983 ± 27	0.512732 ± 17	18.913	15.569	38.844

Chemical and isotopic techniques are as described elsewhere^{38–40}. SiO₂ (samples 1–10) by XRF, K₂O, Rb, Sr and Ba by isotope dilution; SiO₂ analyses of samples 11 and 12 are from ref. 5. Abundances are reported on an anhydrous basis. Sr and Nd isotope ratios are reported relative to 0.708000 for E + A standard, and 0.512640 for BCR-1; 2σ precision refers to least significant figures. Pb isotope data corrected for fractionation, ratio by ratio, using results for NBS SRM 981; absolute values adopted for this standard (16.9373, 15.4925, 36.7054) are from ref. 41. Reproducibility of Pb ratios is better than 0.03% per AMU; in-run precision is a factor of 5 better. Samples 1, 3–7 also analysed for ⁸⁷Sr/⁸⁶Sr after HCl leaching, with identical results to above values.

* Samples 1–7 are from Punta Ingles unit, 8 and 9 from Punta Larga unit, Robinson Crusoe island (Juan Fernandez group), arranged in order of increasing age. Samples 10 and 11 are from San Felix Island; 12 is from San Ambrosio Island.

† A, alkali; B, basalt; O, olivine; T, transitional; Bs, basanite; Te, tephrite.

canos¹²⁻¹⁵. Alteration by weathering may have affected alkali-element abundances in some samples notably numbers 3 and 6, which are characterized by abnormally high K/Rb ratios (Table 1).

Samples from Robinson Crusoe display relatively narrow ranges in $^{87}\text{Sr}/^{86}\text{Sr}$ (0.70351–0.70378), $^{143}\text{Nd}/^{144}\text{Nd}$ (0.51282–0.51288) and $^{206}\text{Pb}/^{204}\text{Pb}$ (19.04–19.21) (Table 1), and lie close to the mantle plane of Zindler *et al.*⁴. The two samples from San Felix are characterized by relatively higher $^{87}\text{Sr}/^{86}\text{Sr}$ and lower $^{143}\text{Nd}/^{144}\text{Nd}$, whereas the San Ambrosio sample is intermediate between San Felix and Robinson Crusoe (Table 1, Fig. 2a). With regard to their Pb isotopic compositions, samples from San Felix, San Ambrosio and Robinson Crusoe show a moderate Dupal signature³, with relatively high $^{207}\text{Pb}/^{204}\text{Pb}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ ratios for a given $^{206}\text{Pb}/^{204}\text{Pb}$ ratio (Fig. 3). The San Felix samples plot well below the Nd–Sr mantle array, whereas basalts from Robinson Crusoe plot approximately within the mantle array, although on the low side (Fig. 2a). Other volcanic samples which plot below the Nd–Sr mantle array include basalts from St Helena¹⁶, the New England Seamounts¹⁷, Tubuaii¹⁸ and various volcanics from continental settings such as the Leucite Hills¹⁹, the Permian Oslo Rift²⁰ and the Scottish Tertiary basalts^{21,22}.

Contamination of mantle-derived magmas at relatively shallow levels by old, granulitic crustal materials^{21,22} tends to produce similar deviations to the low side of the Nd–Sr oceanic array, but this is not a likely explanation for islands in an oceanic setting such as San Felix. Sources for oceanic islands with radiogenic Pb signatures such as San Felix and St Helena cannot have been produced by recycling of upper continental crust into the mantle, as this would displace the data along or to the right of the Nd–Sr mantle array²³ (Fig. 2a). An alternative hypothesis, similar to those of Vollmer *et al.*¹⁹ for the Leucite Hills volcanics and of Menzies and Wass²⁴ for the Kiama xenoliths, suggests that sources of volcanics plotting below the Sr–Nd mantle array were created during an ancient mantle enrichment event in which depleted upper mantle was metasomatized or modified by light-rare-earth-element-enriched, Rb-poor agents. In the case of islands with radiogenic Pb signatures, this metasomatizing agent would also need to have high U/Pb ratios.

The number of 'components' proposed for oceanic mantle basalt sources has steadily increased over the past few years; at least four now seem to be needed^{25,26}, and as many as five have been proposed^{2,27}. Although there is some agreement as to the isotopic signatures of these components, their identification with reservoirs of specified genesis is still controversial. The depleted upper mantle (DMM²⁶) is generally acknowledged to be the source region for mid-ocean-ridge basalts (MORB). The St Helena-type component (HIMU²⁶) is variously identified with ancient subducted oceanic crust^{4,27,28}, metasomatized mantle²⁵ or lower mantle which has lost Pb to the core²⁹⁻³¹. The other two necessary components have enriched isotopic signatures ($\epsilon_{\text{Nd}} < \text{bulk earth}$): one, represented by the Walvis Ridge³² is called EM I²⁶; the other¹⁶, represented by Samoa and the Society islands, is called EM II²⁶ (see Fig. 2). These enriched mantle sources have been variously ascribed to mantle metasomatism^{31,33}, subduction of sediments or continental crust^{16,23}, subduction of altered oceanic crust^{27,28} or delamination of subcontinental lithosphere³⁴. Other reservoirs, with characteristics intermediate to the above four may exist as well (such as primitive undepleted mantle^{35,36}).

As discussed above, the San Felix data extend unusually far below the Nd–Sr array (Fig. 2a) and might be interpreted as evidence for a new mantle component, or as evidence that EM I is an end-member to San Felix, rather than to Walvis Ridge. However, the Sr–Pb plot (Fig. 2b) clearly shows that San Felix does not have $^{206}\text{Pb}/^{204}\text{Pb}$ ratios suitable for EM I. Rather, it is likely that San Felix is itself a mixture of EM I- and HIMU-type sources. Hart *et al.*³⁷ have shown that the lowest-Nd samples from Tubuaii, St Helena, New England seamounts, Comores,

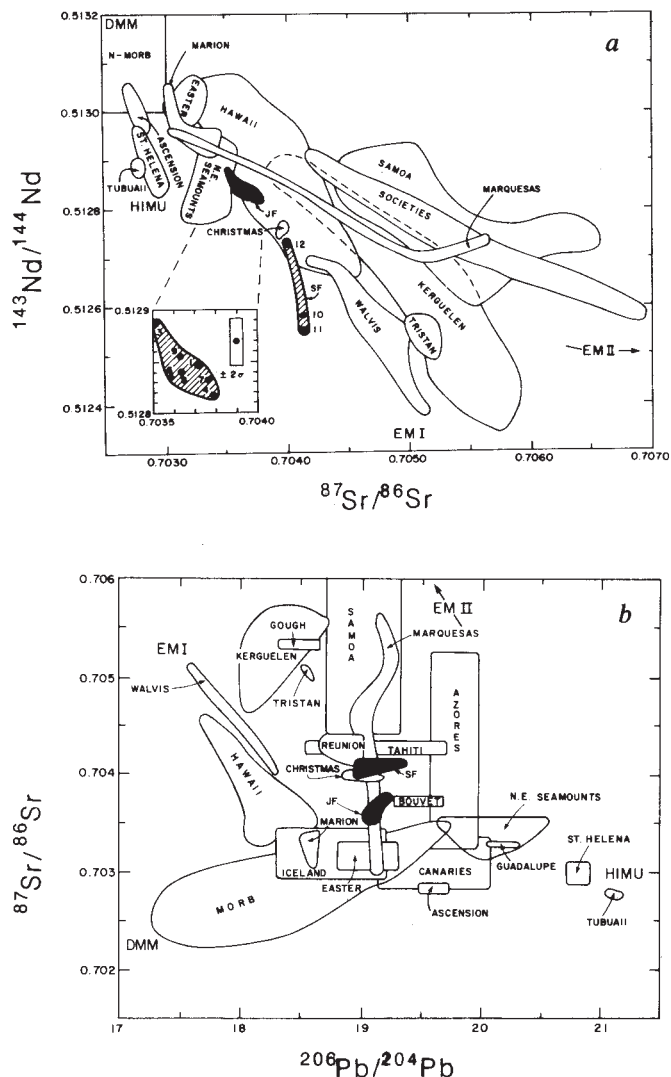


Fig. 2 Nd–Sr (a) and Sr–Pb (b) isotope plots showing San Felix, San Ambrosio and Robinson Crusoe basalt data in comparison with fields for other oceanic island basalts (see refs 2–4). Sample numbers as in Table 1. JF, Juan Fernandez field (individual samples shown in expanded inset in a); SF, San Felix/San Ambrosio data; MORB, mid-ocean-ridge basalts. Locations of four possible end-member mantle components (DMM, HIMU, EM I, EM II) are from ref. 26; see text for discussion. In a, note the linear arrangement of the lowest extensions of the fields for Tubuaii, St Helena, New England seamounts, San Felix and Walvis Ridge. These low-Nd samples also lie on a line in b.

San Felix and Walvis Ridge define a single linear vector in Sr–Nd–Pb space, originating on the mantle plane⁴ near Tubuaii and extending downwards to Walvis. This group of islands shows intra-island isotopic arrays which do not lie along this HIMU–EM I vector, but which extend upward from it toward the mantle plane (though not toward any common end-member on the plane). The implication is that each of these island suites contains a pure (though variable) mixture of HIMU and EM I, and that this mixing preceded the mixing with other components which generated the observed intra-island arrays. In this context, the Juan Fernandez basalts, which lie close to the mantle plane, represent the second mixing end-member of the San Felix–San Ambrosio array. Although this does not settle the question of how the various mantle components came into being, it does suggest that EM I and HIMU are contiguous and related in an evolutionary or kinematic sense, relative to the EM II and DMM components. One scenario would place the EM I and HIMU

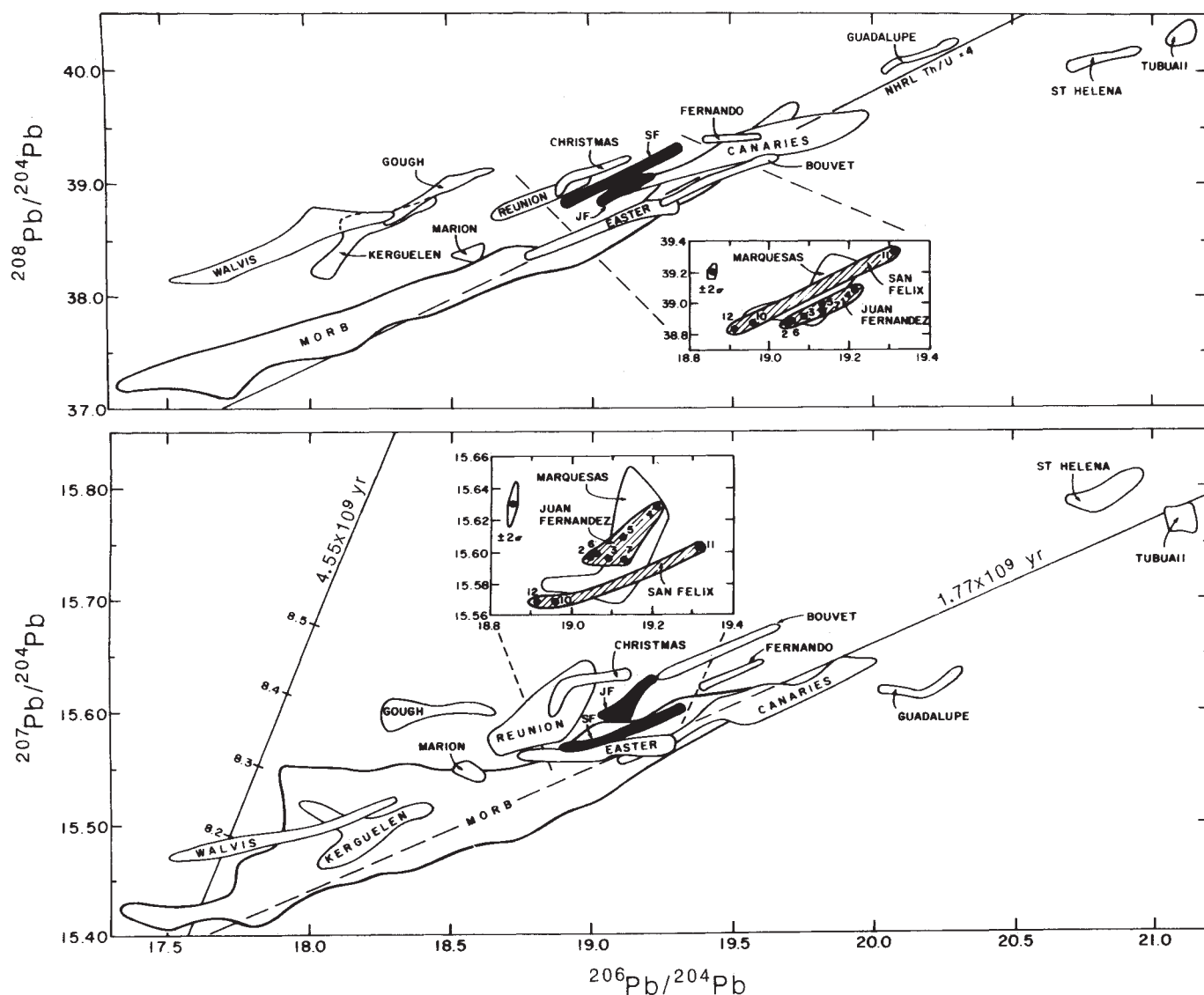


Fig. 3 Pb-Pb-Pb isotope plot showing San Felix, San Ambrosio and Robinson Crusoe data in comparison with fields for other oceanic islands (refs 2-4). JF, Juan Fernandez; SF, San Felix/San Ambrosio; individual data points are shown in expanded insets, with sample numbers as in Table 1; 2σ precision estimates are also shown. The Northern Hemisphere reference line (NHRL) of Hart³ is shown for reference, as is a $4.55 \times 10^9 \text{ yr}$ geochron (with single-stage μ values indicated at tick-marks). Note that the San Felix/Juan Fernandez data overlap with the data for the Marquesas Islands¹⁸, both in this plot (inset) and in Fig. 2b; however, no overlap exists in Fig. 2a. As in Fig. 2, the low-Nd island samples are also linearly aligned in Pb-Pb-Pb space, (the New England seamount data is not shown on this plot, to avoid graphic confusion). This Tubuaii-Walvis array in fact defines a remarkable 'cross-array' on a $^{208}\text{Pb}/^{204}\text{Pb}$ - $^{206}\text{Pb}/^{204}\text{Pb}$ plot, which also includes the fields of a number of 'normal'-Sr-Nd islands.

components in the subcontinental lithosphere; delamination of this lithosphere, followed by mixing with other mantle components, could provide the mixing chronology outlined above³⁷.

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Bacterial scavenging of Mn and Fe in a mid- to far-field hydrothermal particle plume

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The horizontally advected plumes which originate from buoyant hydrothermal fluid discharges and subsequently mix with entrained ambient sea water have been detected hundreds of kilometres from their vent sources by distinctive chemical hydrothermal tracers such as ^3He , Mn and Fe (refs 1–6). Non-conservative plume tracers, such as Mn and Fe, undergo dynamic oxidation–precipitation reactions as the plume is advected away from the vent sources^{1,4,7,8}. Although some of these transformations may be mediated by microbial activity^{9,10}, hydrothermal plumes have largely escaped microbiological examination beyond tens of metres from their vent origins^{11,12}. Microbe–metal interactions in a hydrothermal plume were specifically addressed during our recent expedition (Vent-Plume '85) to the southern Juan de Fuca Ridge (SJFR). The early results, reported here, provide strong evidence of major microbiological influence over Mn scavenging and particulate-Mn distributions.

Microbiological studies of deep-sea hydrothermal vents have been concerned primarily with defining the metabolic processes involved and measuring the magnitude of chemosynthetic productivity (for a review see ref. 13); only a few studies have addressed microbe–metal interactions^{11,14}. Nearly all of these studies have been confined to the immediate vicinity of the vents. Recently, however, far-field hydrothermal influence has been suggested in bacterial capsule–metal (Mn and Fe) interactions within 50 km of the East Pacific Rise (EPR) at 20° N (ref. 9). Even more recently, elevated microbial biomass has been reported in hydrothermal plumes 100 m above the SJFR vent field¹⁰.

In the present study, the extensive plume (see Fig. 1) emanating from the SJFR axial valley, an area of documented hydrothermal activity^{15–18}, was tracked in real time with a sensitive electronics package⁶; a rosette of 30-litre Niskin sample bottles was used to sample the plume selectively. Sea water was collected within the plume vertical maximum at distances of 0.9–8.2 km west of the SJFR axial valley, as well as from a vertical cast taken 4.5 km west of the valley (Fig. 1). Samples were analysed for numbers of metal-depositing and total bacteria (Fig. 2A, B; refs 9, 19) and total particulate Fe and Mn (ref. 20); individual particles were also analysed by transmission electron microscopy–energy dispersive X-ray spectrometry (TEM–EDS⁹). The TEM–EDS analyses show that most of the total suspended particulate Fe is present as amorphous hydrous Fe-oxide precipitates (Fig. 2C, D, 3); thus, estimates of relative background Fe-precipitate abundance were made from TEM sections.

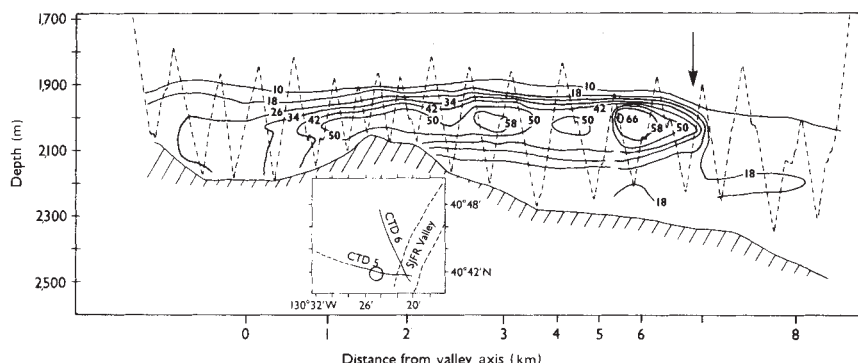
Measurements of total suspended matter (TSM) concentrations indicate the depth of the well-defined plume maximum (2,050–2,150 m) at 4.5 km from the nearest hydrothermal source (Figs 1, 3A). Temperature (not shown), dissolved Mn, and particulate Fe anomalies verified the hydrothermal origin of the plume (Fig. 3A). Maxima in the numbers of total bacteria (Fig. 3A) and in two independent biochemical indicators of biomass (ATP and lipopolysaccharides; C. Winn and J.P.C., unpublished data) clearly coincide with the plume depths. The vertical profile of bacterial capsule numbers is less consistent than, but similar to, the corresponding particulate Mn profile: both show generally elevated values at plume depths, increasing towards the bottom (Fig. 3B). Capsules are distinguished by the tremendous surface areas of the extracellular polymer matrices and by the heavy-metal deposits associated with them (Figs 2A, 3A; refs 9, 21).

A horizontal profile through the off-axis plume maximum showed consistently high particle and Fe-precipitate concentrations up to 6.9 km away from the axial valley, beyond which they decline rather sharply (Fig. 3C). Capsule numbers remain high out to a distance of 7.5 km, with a possible gradual decline moving off-axis. Capsule numbers in the first 7.5 km correspond to the maximum values of the vertical profile taken at 4.5 km from the axis (Fig. 3B).

X-ray microanalysis of individual particles revealed large deposits of Mn and Fe associated with the bacterial capsules (Fig. 4A). No other type of plume particle examined so far shows TEM–EDS-detectable (>1,000 p.p.m.) Mn (Fig. 4A). Microanalysis of the common Fe precipitates showed that large concentrations of Mn are not incorporated during the spontaneous chemical precipitation of iron. Furthermore, capsule Mn deposits increase with distance from vents, whereas Fe deposits decrease (Fig. 4A). Such trends are consistent with the total particulate Fe and Mn data (Fig. 3C). These results, together with the high concentration of capsules in the plume, indicate that the bacterial capsules effectively scavenge hydrothermal Mn and thereby influence the partitioning of Mn in the plume.

Radiotracer experiments (Fig. 4B) and elevated microbial

Fig. 1 Total suspended matter (TSM) isopleths (in $\mu\text{g l}^{-1}$) for CTD 6 horizontal tow; the horizontal axis represents the perpendicular distance from the valley axis. The sawtooth pattern (dashed) shows the tow-path. The inset shows the relative orientation of CTDs 5 and 6 and the axis of the SJFR (the circle indicates the approximate location of the 4.5-km vertical profile shown in Fig. 3A, B). The arrow marks the plume lateral boundary, and corresponds to similar arrows in Fig. 3C, D. TSM values were derived by the normalized conversion of relative light-scattering values.



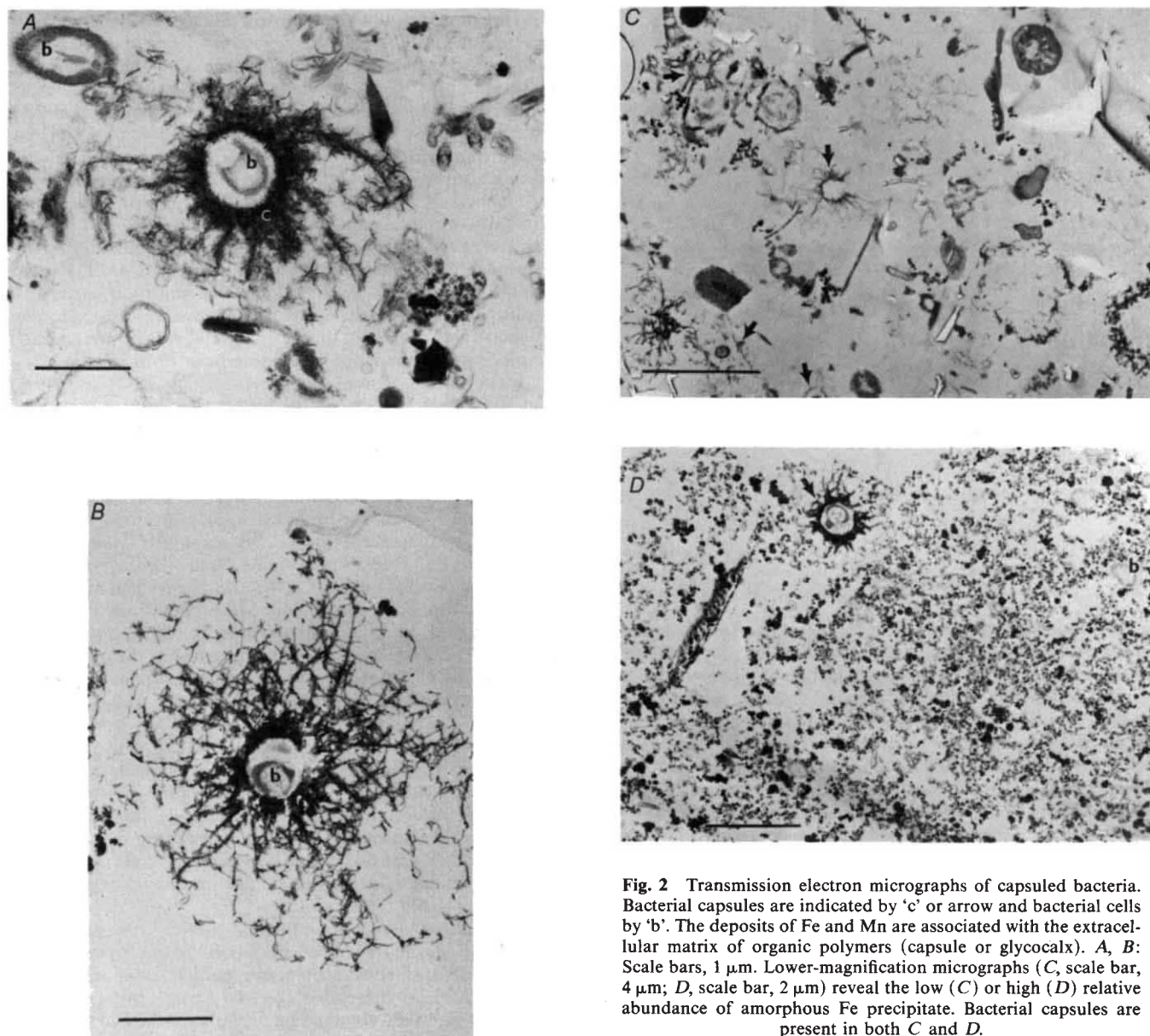


Fig. 2 Transmission electron micrographs of capsuled bacteria. Bacterial capsules are indicated by 'c' or arrow and bacterial cells by 'b'. The deposits of Fe and Mn are associated with the extracellular matrix of organic polymers (capsule or glycocalyx). A, B: Scale bars, 1 μ m. Lower-magnification micrographs (C, scale bar, 4 μ m; D, scale bar, 2 μ m) reveal the low (C) or high (D) relative abundance of amorphous Fe precipitate. Bacterial capsules are present in both C and D.

biomass at plume depths suggest that microbial metabolic activity enhances Mn scavenging. Ehrlich¹⁴ has found that some vent microbial mat bacteria exhibit Mn^{2+} oxidizing activity which is associated with cell envelopes and is peculiar to bacteria from vent environments. We do not know whether such activity extends to the capsules discussed here. Alternatively, the observed metal deposition may be non-enzymatic or proceed from some form of metabolic involvement to a predominantly inorganic process.

On the other hand, capsules probably do not have a major role in Fe scavenging or particulate Fe distributions within the plume extending from the axial valley to at least 7 km off-axis. The dominant presence of the amorphous hydrous Fe-oxide precipitates overwhelms the capsule Fe contributions in this portion of the plume. The absence of significant metabolically enhanced ^{59}Fe uptake (Fig. 4B) reflects this. Unfortunately, the radiotracer experiments cannot be extended to elucidate the specific mechanism of Fe deposition onto the bacterial capsules. Nevertheless, we postulate that the structural and chemical nature of the capsules themselves may be responsible for the

deposition^{9,22}. The additional involvement of continuously synthesized or recycled enzymes, chelators or other Fe-binding factors may be unnecessary. The occurrence in sediments of a high percentage of empty capsules with elevated Fe and Mn deposits relative to suspended capsules adds indirect evidence in support of passive or surface-enhanced metal deposition⁹.

The origin of bacteria, and especially capsuled bacteria, in hydrothermal plumes is not clear. Greatly elevated biomass has been found in the emitted vent fluids at the Galapagos vent¹². However, Lupton *et al.*²³, using hydrographic data to characterize the origin of the hydrothermal plume which resides ~200 m above an active hydrothermal field on the Endeavor segment of the JFR^{24,25}, estimated that the plume layer was composed of at least 70% entrained water which had been transported from deeper in the water column. Capsuled bacteria appear to be enriched in near-bottom waters and surface sediments⁹. Furthermore, Fe- and Mn-encapsuled bacteria are common in microbial mats surrounding hydrothermal vents¹¹. It is conceivable, therefore, that the high numbers of capsuled bacteria found in the mid- to far-field plume of the SJFR originated, in part, from

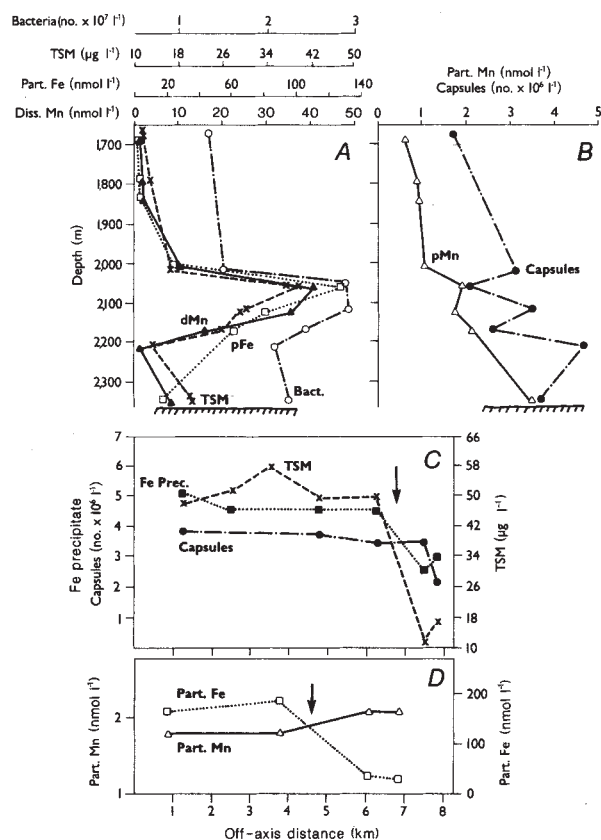


Fig. 3 *A, B*, Data from a vertical profile taken 4.5 km west of active hydrothermal vent fields, SJFR (40° 42.4' N, 130° 26.1' W), including: *A*, bacteria numbers, total suspended matter, particulate Fe and dissolved Mn; and *B*, capsule numbers and particulate Mn. *C*, Data from the plume maximum along tow-path CTD 6 include capsule numbers, Fe-precipitate relative abundance and particle concentration (TSM). Particulate Fe and Mn from tow-path CTD 5 are given in *D*. Arrows in *C* and *D* indicate the off-axis distance at which the respective tows intersect the plume lateral boundary, as indicated by the arrow in Fig. 1 in the case of CTD 6. Open and closed circles, bacteria and capsule numbers, respectively; open and closed triangles, particulate and dissolved Mn; open and closed squares, total particulate Fe and amorphous Fe precipitate; crosses, TSM concentrations. Bacterial numbers were obtained by filtering 60–100-ml subsamples through black Nucleopore filters (0.2 µm, 13 mm) and using epifluorescent (DAPI) direct counting methods; standard errors for 20 counting fields were 3–6%. Ratios of capsuled bacteria to total bacteria were determined by TEM. Bacteria numbers (DAPI) and capsule/bacteria ratios were determined from the same sample collection bottle for each data point. Capsule numbers were calculated by multiplying bacteria numbers by the ratio of capsules to total bacteria⁹; TSM concentrations were derived from light-scattering values (attenuation); and relative Fe precipitate abundance was estimated from TEM samples.

the entrainment of near-bottom waters in the vicinity of the vents. Subsequently, deposition of Mn onto the entrained capsules could proceed within the dissolved-metal-rich plume waters. The latter model would also help to explain the overall vertical profiles for capsules, but not for uncapsuled bacteria. The origin of the latter could be the original emitted vent waters¹² or *in situ* activity.

Our data provide strong evidence of major microbiological contributions to the scavenging of Mn and particulate Mn distributions throughout off-axis hydrothermal particle plumes. The microbes provide exceptionally large and chemically suitable surface areas (the capsule) for metal deposition. There is also

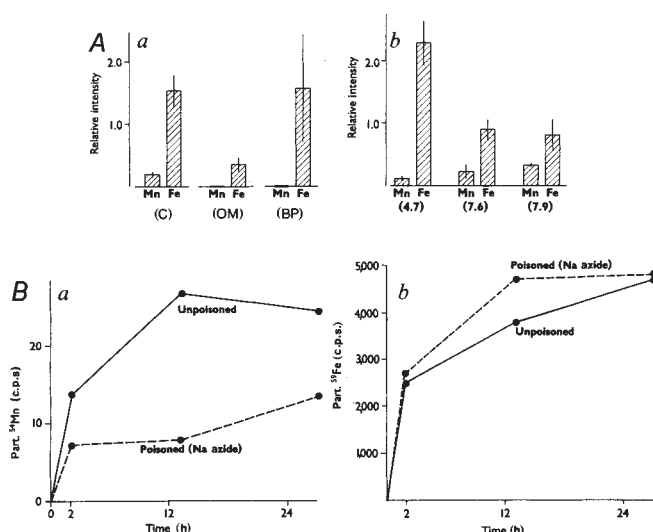


Fig. 4 *A*, Analysis of individual particles from hydrothermal plume maxima by micro-elemental analysis (TEM-EDS spectrometry); samples used were left completely unstained and analysed with a LINK EDS interfaced with a JOEL 100C STEM. *a*, Average relative Mn and Fe peak intensities for capsules (C), other minerals (OM) and background Fe precipitate (BP); *b*, capsule metal deposits at three distances (4.7, 7.6 and 7.9 km) west of the SJFR axial valley; error bars show ± 1 s.e. *B*, Large-volume (6.5 litre) ⁵⁴Mn and ⁵⁹Fe uptake experiments using water from the hydrothermal particle plume maximum at 4 km west of the SJFR. Experiments were run in the dark at 2–4 °C; large (2-l) volumes were used for each subsample, providing highly representative samples of filtered particles. *a*, Particulate ⁵⁴Mn (in counts per s) versus time; *b*, particulate ⁵⁹Fe versus time. Note difference in vertical scales.

evidence that microbes may have a significant direct metabolic role in Mn, but not Fe, scavenging.

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Genetic selection for reproductive photoresponsiveness in deer mice

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Seasonal breeding is common in mammals, particularly in habitats outside the tropics. Climate and availability of food are the ultimate factors that usually dictate the optimal time of year for a mammal to breed; however, day length (photoperiod) often serves as the proximal cue to signal the onset or cessation of seasonal reproduction¹. Some individuals in some populations of deer mice are reproductively responsive to photoperiod, while other individuals in the same population are not. As shown here, selection can dramatically alter the frequency of photoresponsiveness in a laboratory population in only two generations. To our knowledge this is the first demonstration of selection for reproductive photoresponsiveness in any mammal. By implication, some wild populations of deer mice must use multiple, genetic-based reproductive strategies, and the degree to which each such strategy is exhibited must be subject to rapid change in response to both seasonally and momentarily changing climatic and dietary conditions.

In the northern United States and southern Canada, members of the rodent genus *Peromyscus* usually exhibit a well-defined breeding season. Reproduction is largely limited to the spring and summer when food is plentiful, the climate benign and photoperiod long^{2,3}. Several northern populations of *Peromyscus* have been tested in the laboratory for reproductive responsiveness to photoperiod. Using similar techniques it has been found that reproduction is inhibited by short (winter) day lengths in most but not all of the individuals in these populations⁴⁻⁸. This variation could be attributable to experiential factors encountered either prenatally or postnatally⁹⁻¹¹, or it could have a genetic basis. We explored this question by determining the degree to which reproductive photoresponsiveness could be manipulated by artificial selection.

Our test animals were derived from the third generation of a breeding colony of deer mice (*Peromyscus maniculatus nebrascensis*) originally collected near Rapid City, South Dakota (44° N, 103° W). The heterogeneity of this colony's response to photoperiod has been established previously: all individuals mature and breed on long day lengths (light:dark cycle of 16 h:8 h) but only some individuals reach sexual maturity and breed on short day lengths (light:dark = 8:16)⁴.

To begin our selection experiment, 40 pairs were mated on long day lengths, then the females were exposed to short day lengths early in pregnancy. The offspring of these females were defined as the parental generation, and they were weaned at 21–23 days old, maintained one per cage, and the sexes segregated in different animal rooms. The animals were maintained on short day lengths until 8 weeks of age, when their reproductive maturity was assessed. Females were laparotomized, their uteri were recorded as either infantile or partially or fully developed, and their ovaries were examined for corpora lutea as evidence of pubertal ovulation. Testes of males were measured externally using calipers⁸. In each case only completely infantile or fully mature individuals were retained as breeding stock for the next generation.

After 10 weeks on long day lengths to allow sexual maturation of the animals that had been 'suppressed' by short day lengths, two genetic lines were initiated. A reproductively photoresponsive line was begun by mating animals that had remained infantile when reared on short day lengths, and a non-photoresponsive line was begun by mating animals that had matured despite being raised on short photoperiods. Using similar procedures

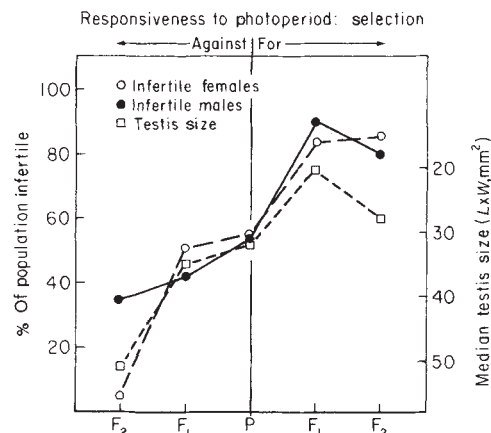


Fig. 1 Change in the frequency of reproductive photoresponsiveness in a laboratory population of deer mice after two generations of selection for and against this trait. The parental generation (P) consisted of 194 individuals of both sexes; 71 of these animals were discarded when they showed intermediate levels of reproductive development at 8 weeks old. Our non-photoresponsive line was begun with 24 pairs, which produced 68 offspring, of which 16 pairs were selected to produce the 43 individuals in the F₂ generation. Our photoresponsive line also was begun with 24 pairs, which produced 83 offspring, of which 16 pairs were selected to produce the 47 individuals in the F₂ generation. L, Length; W, weight.

the resulting two F₁ generations were again directionally selected to produce two F₂ generations.

Figure 1 shows that exposure to a short photoperiod inhibited the reproductive development of about half of the parental generation, while remaining animals were unresponsive to this cue. After two generations of selection only 5% of the females in the non-photoresponsive line had infantile ovaries and uteri when examined at 8 weeks old, as opposed to 86% in the responsive line ($P < 0.001$, χ^2). Comparable figures for males with infantile testes were 35% and 80% for the nonresponsive and responsive lines, respectively ($P < 0.01$, χ^2). Median testis size at 8 weeks of age also was changed markedly by selection ($P < 0.001$, median test).

The divergence seen in Fig. 1 confirms a genetic basis for at least some of the variation in reproductive photoresponsiveness that has been observed in deer mice. Indeed, the rapidity with which our two experimental lines diverged suggests that this trait has a high heritability. Two facts argue against the possibility that selection for rate of reproductive development itself was a factor in our experiment¹². First, our assessments of reproductive maturity were made at 8 weeks of age, which is well after the normal onset of fertility in this species. Second, during our selection experiment we always eliminated all animals showing an intermediate level of reproductive development.

Northern populations of deer mice are routinely and predictably exposed to severe winters in their natural habitats. In theory, a rigid use of photoperiod to limit breeding to the spring and summer months would seem to offer a great advantage in this region. Strict reliance on photoperiod would avoid wasted reproductive effort during the normally harsh winters; also, it would allow a long period of metabolic preparation for a short and intense breeding season. Further, it would enforce the many behavioural and physiological adjustments necessary for survival during harsh winters (for example, food hoarding, moulting, metabolic change¹³⁻¹⁵). Nevertheless, occasional winter breeding has been recorded at surprisingly high latitudes in deer mice¹⁶⁻¹⁸; this seems to occur either during climatically benign winters, or when some individuals are able to locate energetically favourable microenvironments characterized by an abundant food supply. Obviously, such breeding is done by animals that are unresponsive to short photoperiods.

Against this background then, our results suggest that selection for and against reproductive photoresponsiveness must be an exceptionally dynamic process in *Peromyscus*. Many northern populations of this genus must be composed of two or more phenotypes that vary genetically in their reproductive responsiveness to photoperiod. Animals that are not responsive to short day lengths could breed in the summer, but they would probably suffer high mortality during a typically harsh winter. On the other hand, should such individuals encounter a mild winter, or should they find a suitable microclimate during a harsh winter, they could reproduce and thereby add their genes to the population at a time when its size is at its lowest point. Thus, one can visualize a continual balancing of these selective forces, with the result being a highly labile trait whose frequency in a population could shift markedly over short periods of time.

The potential for dynamically changing, multiple reproductive strategies within *Peromyscus* populations has been suggested previously¹⁹. The present demonstration of successful selection for reproductive photoresponsiveness—the first in any mammal—reinforces this possibility. Indeed, the use of multiple reproductive strategies within a population is probably not limited to mid-latitudinal populations of *Peromyscus*, and may not even be limited to photoperiodic regulation^{8,20,21}. Most species of small rodents have short life expectancies, and most environments are relatively unpredictable for animals whose lifespans are measured in weeks or months. Reproductive success under such conditions must require great flexibility in reproductive strategies. The prevailing concept that the reproductive traits of a population have been exclusively tailored to

the average, long-term physical and dietary characteristics of its habitat probably is not applicable to many populations of small mammals.

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Hybrid formation between African trypanosomes during cyclical transmission

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Trypanosomes of the species *Trypanosoma brucei* reproduce primarily by binary fission, but the frequency of enzyme electrophoretic variants in natural populations of *T. brucei* has provided indirect evidence for the existence of a sexual cycle^{1–3}. These studies, coupled with studies of restriction fragment length polymorphisms of genes encoding glycolytic enzymes⁴, have also provided evidence for *T. brucei* being diploid. Here we report direct evidence of gene exchange between two different clones of trypanosomes after mixed infection and full cyclical development in the tsetse fly vector.

Two cloned trypanosome populations (247-L and 386AA) were fed, either separately or as an equal mixture, through a membrane to different groups of tsetse flies (see Fig. 1). Flies showing the presence of metacyclic-stage trypanosomes (indicating completion of full cyclical development) were allowed to feed on mice and the resulting bloodstream infections (386AAA, 723C and 247LA) passaged to produce trypanosome lysates. In addition, a single clone (723CA) was isolated⁵ and then re-transmitted through a tsetse fly to yield the trypanosome populations 723CAB and 723CAE (Fig. 1). From an independent mixed transmission of the two parental clones, two further

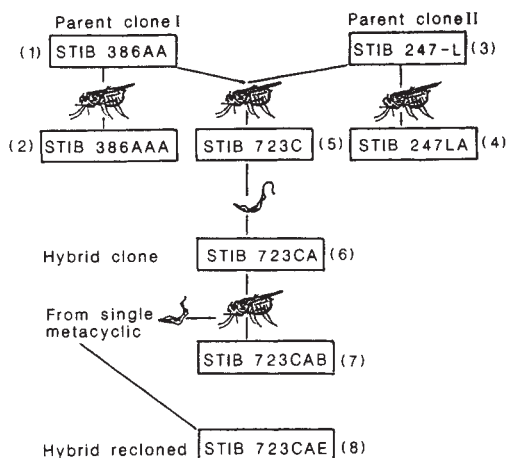


Fig. 1 Pedigree of mixed-infection experiment. Clone STIB 247-L is a derivative of the primary isolate STIB 247, obtained from Coke's hartebeest in the Serengeti National Park, Tanzania, in 1971 (ref. 8). Clone STIB 386AA is derived from TH 114/78 E (020), isolated in 1978 from a patient on the Ivory Coast⁹. *Glossina morsitans centralis* (ILRAD colony) were fed through a membrane on bloodstream trypanosomes from each clone mixed in equal proportions (total of 6×10^7 trypanosomes per ml). Two further groups of *G. m. centralis* were fed at the same time on each of the two clones separately (6×10^7 trypanosomes ml⁻¹). The flies were maintained at 28 °C in 75% relative humidity; they were membrane-fed three times a week on rehydrated pig blood¹⁰. All clones were established using the hanging-drop method of Van Meirvenne *et al.*⁵.

clones (723VI-L and 723VI-M) were derived directly from the metacyclic stage.

The two cloned stocks used in mixed tsetse fly transmission were screened for electrophoretic variation using starch gel electrophoresis or isoelectric focusing (IEF) and were found to differ in five enzymes—phosphoglucosmutase (PGM), isocitrate

dehydrogenase (ICD), alkaline phosphatase (AP), malic enzyme A (MEA) and malic enzyme B (MEB) (Fig. 2). Four of the enzymes show single bands of activity which differ in electrophoretic mobility between the two clones, while in the fifth enzyme (MEB), one clone shows a three-banded pattern and the other a single-banded pattern of identical mobility to the fastest band of the first clone.

The parental clones (numbered 1 and 3 in Fig. 1), the clones derived from separate cyclical transmission of these clones (2 and 4) and the clones derived from mixed transmission (6, 8 and 723VI-L) were screened by starch gel electrophoresis or IEF for the enzymes AP, ICD, PGM, MEA and MEB; Fig. 2 shows the results. The enzyme phenotypes of the parental clones remained unchanged after transmission through the tsetse fly, whereas the enzyme phenotypes of the stocks derived from the mixed transmission were found to differ from those of either parental clone and appeared to be heterozygous for the variant alleles for which the parental clones were homozygous (AP, ICD, PGM and MEA). These results are not due to the presence of mixtures of the parental clones in the tsetse-transmitted progeny, as populations 6, 7 and 8 are clones or directly derived from clones. Thus, mixing of the two parental stocks, followed by transmission through the tsetse fly, results in populations with non-parental phenotypes which are hybrid or heterozygous for parental homozygous markers. Identical results were obtained on analysis of clone 723VI-L, which is derived from a metacyclic form of an independent mixed transmission. These results clearly demonstrate that new, non-parental phenotypes (and therefore genotypes) can be generated by transmission of mixed trypanosome clones through tsetse flies. In addition, the transmission of either parental clone individually or the cloned hybrid progeny (clone 6) results in no alteration of the enzyme markers, implying that individual cloned stocks are stable in terms of phenotype and genotype after tsetse fly transmission.

Probes detecting restriction fragment length polymorphisms (RFLPs) in the genomic DNAs of the parental trypanosome clones were prepared as described in Fig. 3 legend. Southern hybridization of these probes with digests of DNA prepared from hypanosome clones 1, 2, 3, 4, 7, 9 and 10 revealed simple patterns of inheritance. Two probes, pDB9 (Fig. 3a,d) and pBG11 (not shown), revealed that the parental clones are homozygous and differ at the sites detected; the hybrid clones are heterozygous. Probe pBE2 (Fig. 3c) detected a family of fragments generated by *Eco*RI. The hybrid clones inherited all but one of these parental fragments. The results in Fig. 3c are compatible with most of the sites detected in the parental clones being homozygous, and those in the hybrid clones being heterozygous. Examples of markers that are heterozygous in one parent and homozygous in the other have been found using single-copy probes. Among these probes, pBE9 (Fig. 3b,e) detected an instance of the hybrids behaving as homozygotes and not inheriting all the fragments detected in the parents. This result, together with the non-inheritance of the arrowed band in Fig. 3c, demonstrates that the hybrids differ from both parents. The hybrid genotypes have also been detected in trypanosomes isolated from the field, suggesting that they occur naturally.

The DNAs of parental and hybrid trypanosome populations were analysed with DNA probes for variant surface antigen genes (see Fig. 4). The probes are specific for the genes encoding the AnTat 1.1 and 1.8 antigens which have been characterized previously in *T. brucei* stock EATRO 1125 (ref. 6). Both probes, in conjunction with a variety of restriction enzymes (not all shown here), provide restriction patterns that differ between the two parental clones; this result is in keeping with the considerable restriction polymorphism of antigen-specific sequences observed in *T. brucei*⁷. As shown in Fig. 4, the mixed cyclical transmissions gave rise to 'hybrids' with different patterns of reassortment. The pattern revealed in the hybrids by the AnTat 1.1 probe (Fig. 4a) included all the fragments from both parents. The AnTat 1.8 probe (Fig. 4b) revealed a pattern combining all

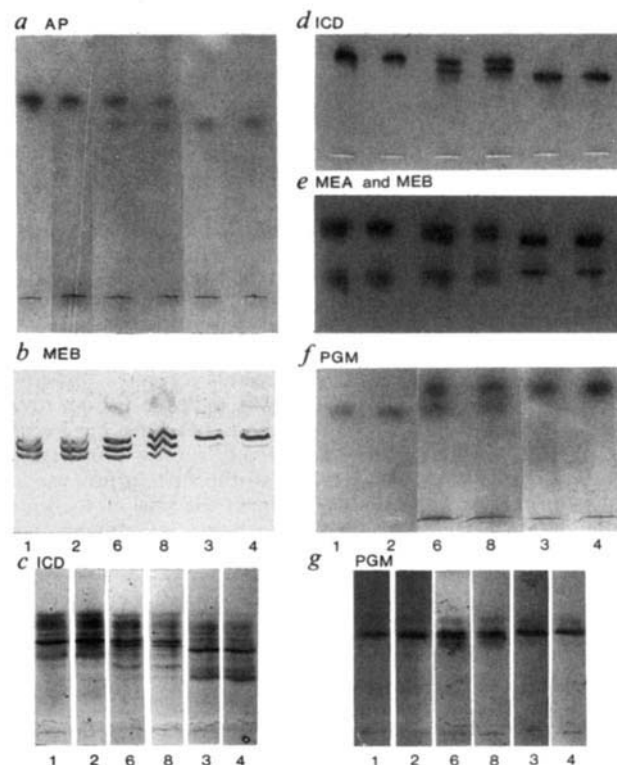


Fig. 2 Starch gel electrophoresis (a, d, e, f) and isoelectric focusing (b, c, g) of extracts of purified trypanosome clones 1–4, 6 and 8. After completion of electrophoresis or focusing, the gels were stained for alkaline phosphatase (AP, a), phosphoglucumutase (PGM, f and g), malic enzyme A (MEA and MEB, e), isocitrate dehydrogenase (ICD, c and d) and malic enzyme B (MEB, b). The methods and buffer systems used for these separations have been described previously^{3,11,12}. The derivation and relationships of the trypanosome populations are shown in Fig. 1.

the fragments from parent 1 with only some fragments characteristic of parent 2. Clearly, some AnTat 1.8 gene family members (indicated by arrows in Fig. 4) were not transmitted to the progeny. The reassorted patterns were conserved through successive clonings and retransmission.

The results presented here directly demonstrate, for the first time, that genetic exchange occurs in *T. brucei*. Clearly, by mixing two trypanosome clones and transmitting the mixture through tsetse flies, trypanosome populations of non-parental phenotype and genotype are produced. With all the markers used, except for the gene probes AnTat 1.8 and pBE9, the progeny derived from mixed transmission of the two trypanosome clones were hybrid or heterozygous for parental markers. The restriction patterns of all these markers would fit a mendelian model of meiosis of the parental strains followed by fusion of a haploid stage, but other interpretations could be proposed to explain the results. The demonstration of genetic exchange in *T. brucei* potentially opens up a new field of trypanosome research—genetic analysis—which should allow the genome to be manipulated. However, considerable further analysis is required to define the basic properties of this new genetic system and to determine whether meiosis occurs; such analysis is currently being undertaken.

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Fig. 3 Detection of RFLPs in parental and hybrid trypanosomes. *a-c*, The results of Southern hybridizations with probes detecting RFLPs in enzyme digests of parental (1-4) and hybrid (7, 9, 10) DNAs. In *d* and *e*, homozygous chromosome fragments are represented by a single line; heterozygous chromosome fragments are shown as double lines. Fragments detected by the probes in Southern blots of *Pst*I-digested trypanosome DNAs are lettered A-F. Heavy bars indicate the cloned fragments used as probes. DNA preparations are numbered as in Figs 1, 2 and 4. *a* Is interpreted in *d* as indicating the presence of homozygous marker fragments in the parental clones, and heterozygous markers in the hybrids; pDB9 detects two fragments in the parental stocks 1-4. Fragment A is detected in trypanosome clones 1-4, fragment B in clones 1 and 2 and C in 3 and 4. The hybrids (7, 9 and 10) are all heterozygous for fragments B and C. In *b* the homozygous marker fragments in clones 7, 9 and 10 appear to have segregated from homozygous $\times$ heterozygous parental combinations (*e*). Probe pBE9 detects two fragments, D and E, in 1, 2, 7, 9 and 10 and an additional fragment, F, in the heterozygous parental stocks 3 and 4. Fragment F is detected because of polymorphism at the *Pst*I site in the cloned sequence. *Pst*I subclones (pBE9-1 and pBE9-2) both hybridize to fragment F. The inheritance of a number of fragments detected with probe pBE2 (*c*). One fragment (arrowed in 1 and 2) is not inherited by hybrid clones 7, 9 and 10.

Methods. Bloodstream-form trypanosomes were transformed to procyclic trypanosomes in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum at 28 °C over irradiated fibroblast feeder layers (2,000 rad; Flow 2002 cells). Procyclic organisms were grown to late log phase in SDM 79 medium¹³, and DNA prepared using standard procedures^{14,15}. Genomic clones were prepared from a reference strain of *T. brucei* (8/18)¹³. Genomic DNA was digested with *Eco*RI and 2.5-5.1-kilobase fragments purified by electroelution from an agarose gel¹⁵. These fragments were ligated with *Eco*RI-digested pJSC73 (ref. 16) and used to transform *Escherichia coli* HB101. Colonies containing plasmids with inserts were detected by the colour selection method¹⁶ and plasmids prepared by a rapid isolation procedure¹⁷. After electrophoresis in 0.8% agarose, the plasmids were transferred to nitrocellulose filters and hybridized with nick-translated total genomic DNA from the reference strain ($1-2 \times 10^8$ c.p.m. per μ g DNA)^{18,19}. Filters were washed three times for 30 min each in $2 \times$ SSC plus 0.1% SDS at 65 °C, and exposed to Fuji X-ray film at -70 °C for up to 5 days, with an intensifying screen. Recombinant plasmids yielding the weakest signals in our conditions identified single- or low-copy number sequences. Southern hybridization¹⁸ of these probes to trypanosome DNAs from parental and hybrid clones digested with different restriction enzymes was used to detect RFLPs; filters were washed under conditions of high stringency ($0.1 \times$ SSC, 0.1% SDS at 65 °C) and autoradiographed.

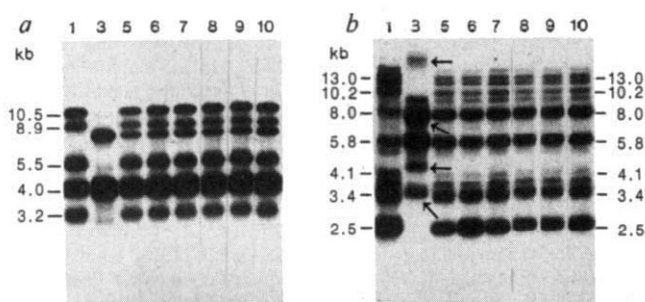
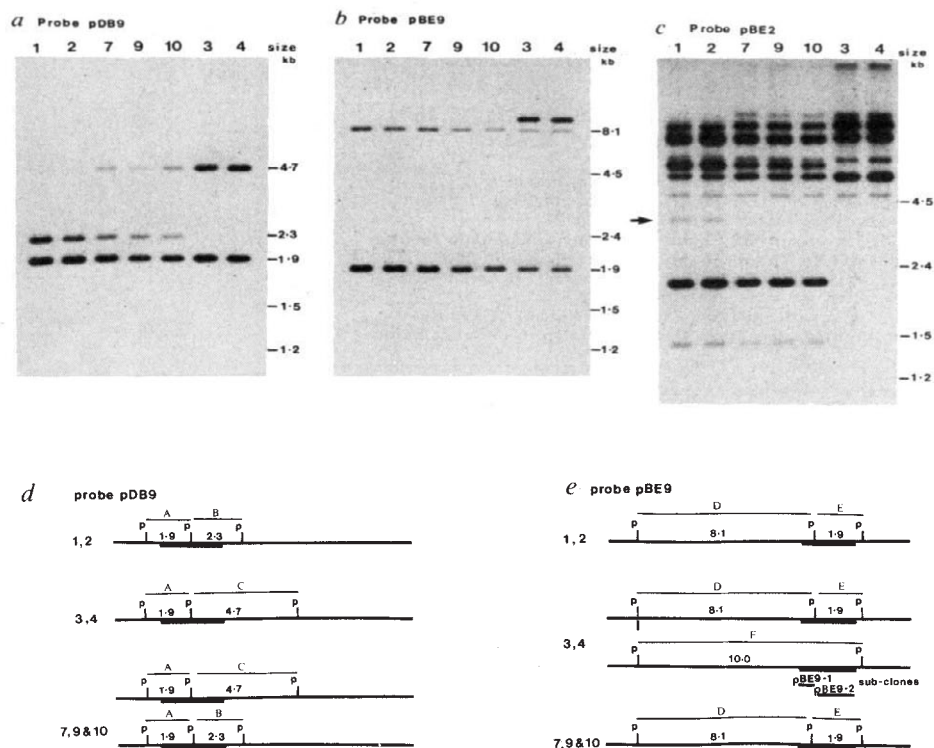


Fig. 4 Detection of restriction fragments specific for the genes encoding the antigens AnTat 1.1 (*a*) and AnTat 1.8 (*b*) in parental and hybrid trypanosomes. The different DNA preparations (1, 3, 5-8) are numbered according to the pedigree shown in Fig. 1. Tracks 9 and 10 contain DNAs from hybrid clones (723VI-L and 723VI-M) obtained in an independent mixed transmission experiment. The antigen-specific fragments of the hybrids (tracks 5-10) appear to be a combination of those of the two parental clones (tracks 1 and 3), except for the arrowed fragments in lane 3, which are not transmitted to the progeny.

Methods. The AnTat 1.8 and 1.1 probes were prepared as described previously⁶, then nick-translated and hybridized to Southern blots of genomic *Pst*I digests, obtained as described in Fig. 3 legend.

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Analysis of human T-lymphotropic virus sequences in multiple sclerosis tissue

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Several observations suggest that retroviral infection is involved in the pathogenesis of the human demyelinating disease multiple sclerosis (MS). First, lymphadenopathy-associated virus/human T-lymphotropic virus type III (LAV/HTLV-III), the agent of acquired immune deficiency syndrome (AIDS), has been shown to be neurotropic in man¹. Second, the genetic organization of the lentivirus visna, which causes a chronic demyelinating disease of sheep, closely resembles that of LAV/HTLV-III². Recently, Koprowski and colleagues reported that MS is associated both with raised levels of circulating antibodies to HTLV-I and with the presence of HTLV-I-specific RNA within cell lines derived from the cerebrospinal fluid (CSF)³. Here we report that no HTLV-I-like or LAV/HTLV-III-like sequences can be detected, by *in situ* hybridization, in central nervous system (CNS) tissues from MS patients, and that no specific HTLV-I-like signal in peripheral blood mononuclear cells or in CSF cell lines is characteristic of MS. Furthermore, enzyme-linked immunosorbent assay (ELISA) analysis of circulating and CSF antibodies for HTLV-I reactivity fails to distinguish between MS and control groups.

Tissue blocks of active white matter lesions from 12 MS patients were selected for the presence of inflammation, macrophage infiltration and active demyelination; in some cases variable gliosis was also present (Fig. 1a). *In situ* hybridization was performed on serial sections, using a probe complementary to the long terminal repeat, *pX*, *gag*, *pol* and *env* regions of the HTLV-I genome, a probe complementary to the entire LAV/HTLV-III genome, and a control pBR322 probe. No specific RNA sequence was detected in any section following autoradiographic exposure times of 1 and 2 weeks.

Circulating mononuclear cells from 18 MS patients and 18 control individuals were separated by density gradient centrifugation, mounted on acetylated slides by cytocentrifugation and fixed. Table 1 shows the results of *in situ* hybridization with the HTLV-I and pBR322 probes. Cells were defined as positive when the number of grains counted was greater than five times background (Fig. 1b). It is noteworthy that both the HTLV-I and pBR322 probes labelled a small number of cells (10^{-4} – 10^{-5} cells counted) in approximately half of both the MS and control samples. The morphology of cells labelled by both probes was heterogeneous and included small cells with scant cytoplasm, presumably lymphocytes, and, prominently, some larger cells with cloven or bi-lobed nuclei (Fig. 1c). Eosinophils, which were occasionally present in some samples, were also labelled by each of the probes; nonspecific labelling of this cell type during *in situ* hybridization has been noted previously⁴; no differences were present between the MS and control populations (Table 1).

CSF lines were derived from three individuals with active MS by stimulation with phytohaemagglutinin-P and by expansion with medium supplemented with interleukin-2 (Biotest). No positively labelled cells were detected in any of the three lines following *in situ* hybridization with the HTLV-I probe (10^5 cells counted per sample) (Table 1). Analysis of peripheral blood

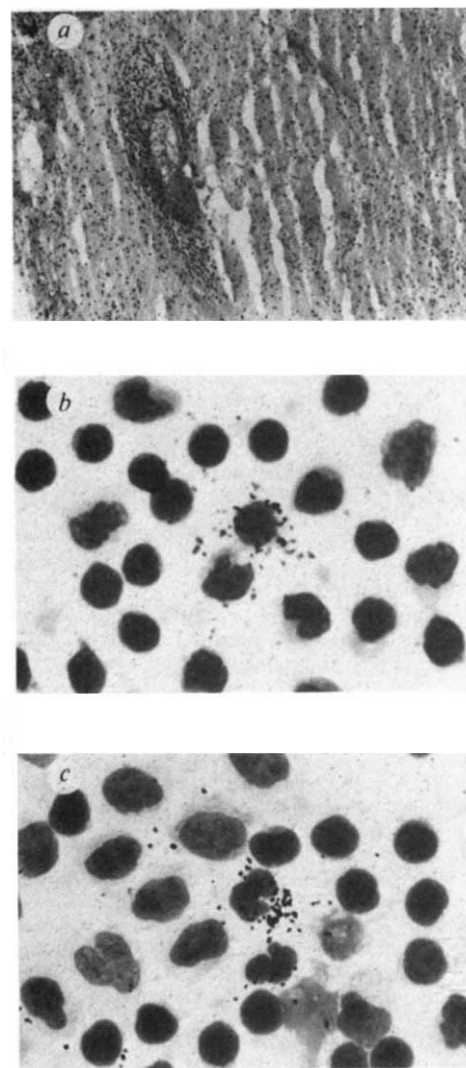


Fig. 1 *a*, Perivascular and parenchymal inflammation is present at the edge of an active MS plaque (haematoxylin-eosin). *b*, *In situ* hybridization of peripheral mononuclear cells with the HTLV-I DNA probe. The figure shows a typical small round cell which was labelled by the HTLV-I probe. *c*, A peripheral blood mononuclear cell with a cloven nucleus is labelled by the HTLV-I probe.

Methods. *a*, For this study, fresh frozen necropsy ($n = 11$) or biopsy ($n = 1$) brain material was derived from patients with clinically definitive and pathologically confirmed MS. The MS material used is part of a larger collection which has been described previously⁶. Serial sections from each frozen tissue block were mounted on pretreated slides^{4,7}, fixed with ethanol acetic acid (3:1 vol/vol), and hybridized *in situ* as described elsewhere, including pretreatments with acid, heat and proteinase K^{4,7}. DNA probes for HTLV-I, LAV/HTLV-III and pBR322 sequences were labelled by nick translation with ³⁵S to a final specific activity of 2×10^5 d.p.m. per ng. After washing, slides were dipped in Kodak NTB-2 emulsion, exposed for 11 days, developed and counterstained with a Giemsa stain. *b*, Cells were prepared by density gradient centrifugation, mounted on microscope slides with a cytocentrifuge and fixed in 0.5% formaldehyde, 0.5% glutaraldehyde, 0.1 M phosphate buffer pH 6.0, 1.6% glucose, 0.002% CaCl₂, 1.0% dimethyl sulphoxide⁸. *In situ* hybridization was performed as described above for brain sections.

mononuclear cells from each of these patients had shown that several positive cells were detected with both the HTLV-I and pBR322 probes at the time that the CSF lines were established.

Serum antibody reactivity against HTLV-I was measured by ELISA, and no differences were found between a larger group of MS and control patients (Fig. 2). Serum from one MS patient was strongly reactive with HTLV-I, a finding confirmed by

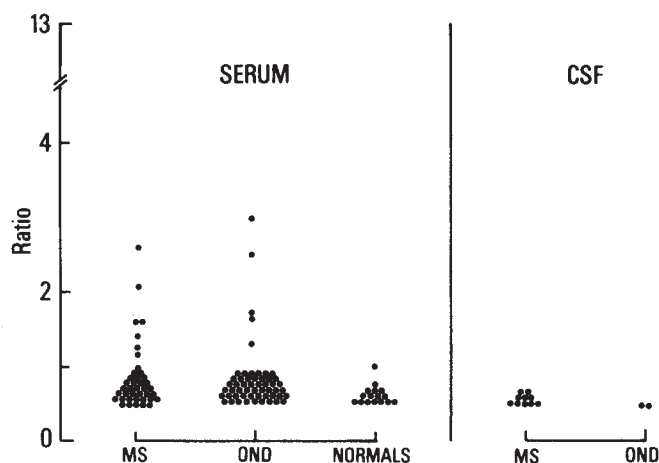


Fig. 2 Distribution of antibodies to HTLV-I in MS patients and controls. Coded serum and CSF samples were tested by the Biotech HTLV-I ELISA test using as antigen semipurified disrupted HTLV-I virions produced by the HUT-102 cell line⁹. The level of positivity was estimated by the ratio of absorbance between the test serum and the average of duplicate negative sera. OND, other neurological disease.

immunoblot analysis. Sera from two other MS patients were weakly reactive by ELISA but negative by immunoblot. Serum from one control, a patient with amyotrophic lateral sclerosis, was weakly reactive against HTLV-I both by ELISA and immunoblot. CSF samples from 10 MS patients were uniformly unreactive to HTLV-I by ELISA (Fig. 2). CSF and brain material was available from one MS patient whose serum was reactive to HTLV-I by ELISA, and in this patient no CSF antibodies or viral RNA in brain tissue was found.

These results fail to confirm a recent report implicating infection with an HTLV-I-like virus in MS³. It is unlikely that this discrepancy is due to a difference in patient selection, as the MS population chosen for our study consisted of patients with both the relapsing-remitting and the chronic progressive forms of the disease and included patients from both Europe and the United States. The present study also represents the first analysis of HTLV sequences in MS brain material and the negative results obtained contrast with the finding that LAV/HTLV-III-specific sequences can be detected by *in situ* hybridization in the brains of some AIDS patients¹, a finding confirmed in our laboratory Vazeux and M.B., in preparation). The absence of circulating antibodies to HTLV-I in this population is also in striking contrast to the consistent findings of one of us (G.deT.) in patients with tropical spastic paraparesis⁵.

These results do not rule out the possibility that a small percentage of MS patients might be found to have an associated HTLV-I infection. In the present study, the one patient (of 42

tested) showing a strong serological response to HTLV-I was of French origin and not distinguishable on clinical grounds from the other MS patients studied. It also remains possible that an as yet undefined retrovirus is present in MS. In this regard, the extensive antigenic and sequence diversity which exists between different retroviruses makes it likely that other methods will be required to identify the presence of such agents in human disease states.

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KOPROWSKI *ET AL.* REPLY ON PAGE 178.

Lack of evidence for involvement of known human retroviruses in multiple sclerosis

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The recent report by Koprowski *et al.*¹ that human T-cell lymphotropic retroviruses (HTLVs) may be involved in the development of multiple sclerosis (MS) has aroused much interest². The report was based largely on immunological evidence, using enzyme-linked immunosorbent assays (ELISAs) with viral antigens or disrupted virions. We have accordingly sought confirmation by screening sera and cerebrospinal fluid (CSF) samples from MS patients against cell lines infected respectively with adult T-cell leukaemia (ATL) virus (ATLV/HTLV-I) of Japanese cells (MT-1 and MT-2 lines), our own isolate from British black patients with ATL, the MoT cell line which produce HTLV-II, and our own T-cell line containing a local isolate of acquired immune deficiency syndrome (AIDS) virus (C-LAV/HTLV-III). We have failed to find antibodies against these retroviruses in the sera or CSF. Furthermore, neither virus could be isolated from the peripheral white blood cells of two MS patients.

We have used the cell-based immunoperoxidase (IP) method, which we developed and found to be a highly sensitive test³ for antibodies to the AIDS virus and to ATL/HTLV-I. In both tests, virus-infected cells which produce large quantities of the viral antigen were used. Each sample was tested in duplicate and also included a control (non-infected cells). The results of the IP reaction could be clearly distinguished by the naked eye

Table 1 *In situ* hybridization of mononuclear cells in multiple sclerosis

Source	Diagnosis	Specific reactivity with HTLV-I*	
		Present	Absent
Peripheral blood	Multiple sclerosis (n = 18)	2	16
	Control individuals (n = 18)†	3	15
Cerebrospinal fluid	Multiple sclerosis (n = 3)	0	3

* Specific hybridization with the HTLV-I probe was defined as those samples in which cells were labelled only with HTLV-I or in which the frequency of cells labelled with this probe was greater than that present with the control pBR322 probe.

† Includes 11 individuals with a variety of non-MS neurological diseases and 7 normal controls.

and verified by examination under conventional low-power microscopy. This allows for the elimination of false readings associated with radioactivity of ELISA counters.

Table 1 lists the origin and clinical state of the sera and CSF and shows that we failed to detect antibodies to the oncoviruses (ATLV/HTLV-I, HTLV-II) or to the lentiviruses (LAV/HTLV-III) in any of the 46 sera and 15 CSF from MS patients.

In our preliminary studies we also tried to isolate a retrovirus from the peripheral white blood cells (WBC) of two patients with MS. The WBC were separated on Ficoll before being divided into two parts. One part was co-cultivated with fresh

Table 1 Results of retrovirus tests

	ATLV/ HTLV-I	HTLV-II	LAV/ HTLV-III
UK: Paired, MS sera	0/15	0/15	0/15
MS CSF	0/15	0/15	0/15
Paired, OND sera	0/20	0/20	0/20
OND CSF	0/20	0/20	0/20
Sweden: MS sera	0/21	0/21	0/21
Germany: MS sera	0/10	0/10	0/10
Japanese ATL sera	5/5	5/5	0/5
British black ATL sera	3/3	3/3	0/3
British homosexuals' sera	0/61	0/10	61/61

MS, multiple sclerosis; CSF, cerebrospinal fluid; OND, other neurological diseases, including suspected but not confirmed MS; ATL, adult T-cell leukaemia.

human cord WBC and the other part was cultured with the Karpas T cells³. If ATL/HTLV-I or HTLV-II were involved in MS, one might expect the cord WBC to become infected and express the viral antigen. In contrast, if an AIDS-like lentivirus was involved, one would have expected cell lysis of both the cord T cells and our T-cell line, or at least development of an antigen that reacted with sera containing antibodies to LAV/HTLV-III or ATL/HTLV-I.

The two cultures of cord cells, and the two cultures of our T-cell line, which were co-cultivated with the WBC from the MS patients, were tested for the expression of ATL/HTLV-I and LAV/HTLV-III after 2 and 4 weeks in culture, using the IP method. Neither the cord WBC nor our T-cell line expressed any antigens that reacted with human sera containing antibodies to ATL or to LAV. Since sera of patients with ATL contained antibodies which cross-reacted with HTLV-II, one would expect these sera to react with HTLV-II-infected cells if a related virus is involved in MS.

In summary, we failed to detect any antibodies against ATL/HTLV-I and HTLV-II or LAV/HTLV-III in the sera and CSF samples from MS patients, nor could either of these viruses be isolated from the WBC. The claim to the presence of antibodies which react with the known human retroviruses is based on ELISA tests. We now know that some of the ELISA systems may give a high rate of false-positive results. The report that 37% of Israeli Falashas were ATL/HTLV-I-positive has turned out to be wrong, probably due to the ELISA method⁴. Similarly, it has been repeatedly reported that some of the ELISA test kits used for AIDS screening give a high rate of false-positives^{5,6}. The results of such ELISA methods might cause premature claims to the involvement of HTLV in MS.

The possible viral aetiology of MS remains enigmatic; should a retrovirus eventually prove to be a pathogenic factor, it is likely to be distinct from the known human retroviruses.

We thank Professor P. Lachmann for providing some of the serum/CSF samples.

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KOPROWSKI ET AL. REPLY: We are pleased that other investigators^{1,2} have undertaken the difficult task of searching for the involvement of retroviruses in multiple sclerosis. However, it is difficult for us to compare the results of our study³ with the data presented by Hauser *et al.*¹ and Karpas *et al.*². The first study used a commercially prepared kit for HTLV-I antibody while the second used visual detection of HTLV-I antibody by immunoperoxidase on infected cells. The drawback of both these assays is that the concentration of the HTLV group-specific (gag) antigen p24 in the kit is unknown and is considerably lower in cells than that used for our antibody determinations by ELISA. Despite this, the results of Hauser *et al.*¹ with MS sera and normal controls are almost identical to the results shown in our Fig. 2³. The discrepancy between our results and theirs with other neurological disease (OND) patients may be explained if we knew which OND patients were tested by Hauser *et al.*¹. While Karpas *et al.*² may find their immunoperoxidase test sensitive enough to detect antibodies in extremely high-titred AIDS⁴ and in eight leukaemia sera, we expect that this assay would not detect low-titred (HTLV-I cross-reactive) antibody found in MS patients. Because of the fluctuation of the HTLV p24 antibody levels, it does not make sense to search with low-sensitivity assays for antibodies in a single sample of either serum or cerebrospinal fluid of MS patients. 'False-positives' in our ELISA were excluded by specific competitive inhibition assays³.

The inability of Hauser *et al.*¹ to detect retroviral sequences by *in situ* hybridization may be due to several factors: (1) use of complementary DNA probes rather than the much more sensitive riboprobes^{5,6}; (2) the stringency conditions under which the test was run (not mentioned by Hauser *et al.*¹ but crucial in our assay); (3) restriction of sensitivity of the test by nonspecific hybridization with pBR322 plasmid alone. Hauser *et al.* provide neither positive controls showing the limitation of the sensitivity of the test nor controls confirming the presence of hybridizable RNA in the lymphocytes.

In the light of the considerable time and effort invested in the isolation of the human retroviruses I, II and III, it is not surprising that Karpas *et al.*² failed to isolate the 'MS virus' from two randomly chosen MS samples in 4 weeks. We also emphasize points repeatedly made in our report that detectable antibodies are: present in low titre; cross-reactive, that is, not reactive specifically against the test HTLV; and, most importantly, present only sporadically in cerebrospinal fluid and sera during the course of disease. Karpas *et al.*² made a point that if HTLV is involved in MS, it is "distinct from the known human retroviruses". We agree and that was precisely our conclusion.

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A functional T3 molecule associated with a novel heterodimer on the surface of immature human thymocytes

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The known T-cell receptors (TCRs) involved in the recognition of antigen and major histocompatibility complex (MHC) molecules are glycoproteins comprised of polymorphic disulphide-linked α - and β -chains¹⁻⁵. The genes encoding these chains are homologous to immunoglobulin genes and consist of V (variable), J (joining) and C (constant) regions that rearrange during development⁶⁻¹³. TCRs are expressed relatively late in thymocyte development and only in association with an invariant molecular complex of proteins termed T3 (refs 3, 14). Immature thymocytes do not express the TCR-T3 complex but do express messenger RNA encoding a third rearranging T-cell receptor-like gene, termed T γ (refs 13, 15-20). Here we report a clone of normal immature T4⁺T8⁺ human thymocytes, designated CII, which does not express mature mRNA for T α or T β genes, but does express high levels of T γ mRNA. This clone also expresses high levels of surface T3, and antibodies to T3 induce immunologically relevant functions in CII cells. Immunoprecipitation of CII surface-labelled proteins with anti-T3 co-precipitates a T3 molecular complex together with two additional and novel peptides of relative molecular mass (M_r) 44,000 (44K) and 62,000 (62K).

To isolate immature T4⁺T8⁺ cells, human thymocytes were treated with anti-T4 and anti-T8 antibodies, and T4⁺ and T8⁺ cells were removed by rosetting with anti-mouse immunoglobulin-coupled red cells^{15,20-22}. The original thymocyte population contains cells expressing the T11, T3, T6, T4 and T8 antigens (Fig. 1a). In contrast, the non-rosetting population lacks T4- and T8-bearing cells but expresses the T11 and T6 surface antigens (Fig. 1b)^{20,23}. The T4⁺T8⁺ cells constituted 3.5% of the total thymocyte population and the number of these cells expressing T3 varied between 0 and 5%. To generate continuous cell lines, T4⁺T8⁺ cells were cultured with interleukin-2 (IL-2) and feeder cells. Over a short culture period (4 days), T4⁺T8⁺ cells lost expression of T6 and 80-90% of the cells expressed high levels of T3 (Fig. 1c). Cloning of these cells yielded several T3⁺T4⁺T8⁺ cell lines, one of which, CII, was studied in further detail. CII remains an IL-2- and feeder cell-dependent cell line which continues to be T3⁺, T4⁺ and T8⁺ (Fig. 1d). We confirmed the T4⁺T8⁺ phenotype of CII by Northern blot analysis. Neither the 3.0-kilobase (kb) T4 mRNA nor the 2.4-kb T8 mRNA was expressed in CII (refs 24, 25 and data not shown).

We next assessed whether the T3 molecule on CII cells was functional. Mature T cells can be activated by anti-T3 antibody in the presence of the phorbol ester 12-*o*-tetradecanoyl phorbol acetate (TPA) to secrete IL-2^{26,27}. When CII cells were stimulated with TPA in the presence of anti-T3 antibody (OKT3), significant amounts of IL-2 were produced (Fig. 2a). In contrast, TPA alone or anti-T11 (OKT11A) and TPA resulted in only minimal IL-2 release. In addition, CII, like mature T cells, could be activated to proliferate by OKT3 in the presence of monocytes²⁸. A monocyte-enriched (E⁻) population was irradiated and added to CII cells in the presence or absence of anti-T3 or control anti-T11 antibody. Anti-T3 but not anti-T11 induced a significant proliferative response which required the presence of E⁻ cells (Fig. 2b). Neither CII cells nor irradiated E⁻ cells

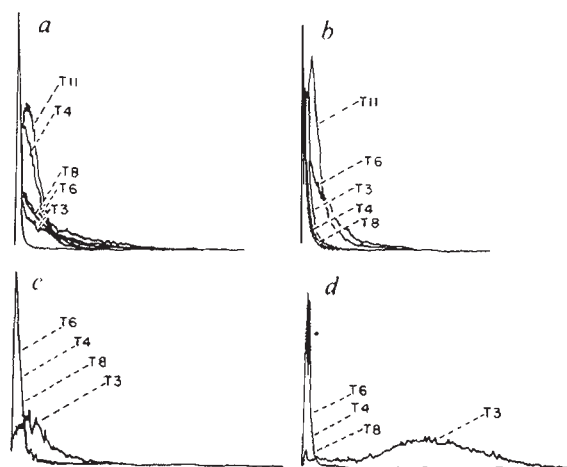


Fig. 1 Cytofluorographic analyses of fresh thymocytes (a), freshly selected T4⁺T8⁺ thymocytes (b), cultured T4⁺T8⁺ cells (c) and the clone CII (d). **Methods.** Thymus tissue was obtained from children 2-11 years old undergoing cardiac surgery, and single-cell suspensions were prepared. To obtain T4⁺T8⁺ cells and the reciprocal T4⁺T8⁺ cells, unfractionated thymocytes were treated for 30 min at room temperature with a combination of OKT4 (1/1,000 of ascites) and OKT8 (1/2,000 of ascites), and rosetted with ox red blood cells coupled to rabbit anti-mouse IgG at a final haematocrit of 2.5%^{24,25}. Rosetting (T4⁺ and T8⁺) cells were separated from non-rosetting (T4⁺T8⁺) cells by Ficoll-Hypaque density centrifugation. The rosetting red cells were lysed with Tris-buffered ammonium chloride to obtain a pure T4⁺T8⁺ population²². T4⁺T8⁺ cells were cultured at 1×10^6 cells ml⁻¹ in the presence of 0.5×10^6 cells ml⁻¹ of irradiated (7,000 rad) B-lymphoblastoid cells, in a final volume of 2 ml in 12-well tissue culture plates (Costar). Recombinant IL-2 (100 U ml⁻¹; Hoffman-La Roche), and 1% phytohaemagglutinin (Gibco) were added. The final medium for all cultures was Iscove's modified Dulbecco's medium (Gibco) supplemented with 1% penicillin-streptomycin (Gibco) and 10% fetal calf serum (Hyclone), and cultures were grown in a humidified atmosphere with 5% CO₂ at 37 °C. After 4 weeks of bulk culture, cells were cloned at limiting dilutions in 96-well round-bottom plates with lymphoblastoid feeders and expanded under conditions similar to those used in the initial bulk culture. For cytofluorographic analysis, 10^5 cells were incubated at room temperature with a 1/2,000 dilution of the appropriate antibodies (OKT11, OKT6, OKT4, OKT8 or OKT3) for 30 min. The cells were then washed and incubated for an additional 30 min at 4 °C with fluorescein-conjugated goat anti-mouse immunoglobulin (G/M FITC) (Cappel Laboratories) and washed. The cells were analysed on a Model 30-H Cytofluorograf (Ortho Instruments). The results shown represent analysis of 10^4 cells for each population as indicated.

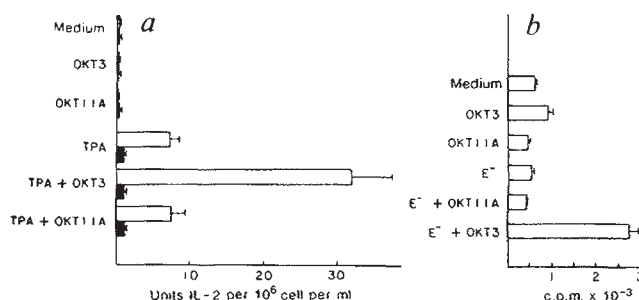


Fig. 2 Activation of CII cells by anti-T3 antibodies to secrete IL-2 and proliferate. **a**, CII cells (open bars) or B-lymphoblastoid cells (closed bars) were washed and placed in 96-well microtitre plates in fresh medium at a concentration of 0.5×10^6 cells ml⁻¹ in a final volume of 200 μ l. TPA (Sigma) was added to the cultures at 5 ng ml⁻¹. OKT3 or OKT11A was added at a final concentration of 1/500 of ascites fluid. After 24 h, the supernatants were collected and added to the murine IL-2-dependent CTLL cell line²⁷. After overnight incubation at 37 °C in a humidified atmosphere with 5% CO₂, the proliferation of CTLL was assessed by ³H-thymidine incorporation over an additional 6 h. A standard reference curve was obtained for each experiment, and the IL-2 units calculated as described previously³⁶. The results shown are expressed as units of IL-2 produced per 10^6 cells ml⁻¹, and represent the mean $\pm$ 1 s.d. of triplicate wells. **b**, CII cells were washed and placed at 1×10^6 cells in a volume of 200 μ l in 96-well flat-bottom plates. E⁻ cells, obtained from normal donor peripheral blood mononuclear cells, were irradiated (2,000 rad) and added at 0.5×10^6 cells ml⁻¹ (ref. 37). OKT3 and OKT11A were added at 1/500 dilution of ascites fluid. Plates were cultured as described previously. Proliferation was assessed after 48 h of culture by ³H-thymidine incorporation³⁷. The results shown represent the mean $\pm$ 1 s.d. of triplicate wells.

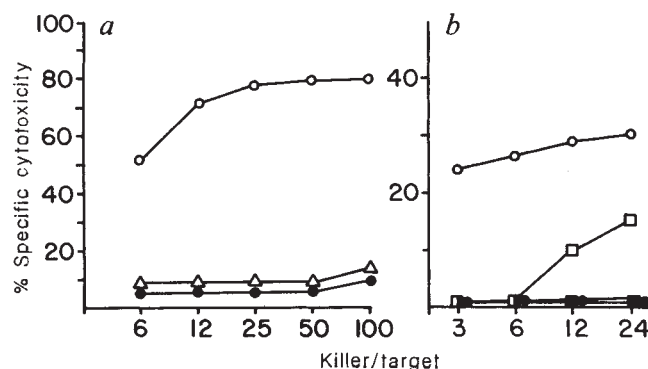


Fig. 3 Induction of cytotoxicity by OKT3 in CII cells. *a*, CII cells were added alone (●), with OKT3 (○) or with OKT11A (△) to ^{51}Cr -labelled U937 cells, and specific cytotoxicity was calculated (y-axis) at the indicated killer/target ratios. *b*, CII cells were added alone (solid symbols) or in the presence of OKT3 (open symbols) to ^{51}Cr -labelled U937 cells (circles) or ^{51}Cr -labelled K562 cells (squares).

Methods. K562 and U937 cells were grown in culture medium containing 10% fetal calf serum under culture conditions described in Fig. 1 legend. For each experiment, 10^5 viable cells were removed from the cultures and treated with ^{51}Cr -sodium chromate (Dupont), washed and placed (5,000 cells per well) in U-shaped 96-well plates (Costar). CII cells were added at the indicated killer/target ratios in a final volume of 200 μl . OKT3 and OKT11A (1/500 final dilution of ascites fluid) were added at the beginning of the assay. After 4 h of incubation at 37°C in a humidified atmosphere containing 5% CO_2 , 100 μl of the spun supernatants were removed and ^{51}Cr content measured³⁷. Specific cytotoxicity was calculated from the formula: specific cytotoxicity = (experimental ^{51}Cr release - spontaneous ^{51}Cr release) / (total ^{51}Cr release - spontaneous ^{51}Cr release). Total ^{51}Cr release was assessed by Triton lysis of the ^{51}Cr -labelled cells.

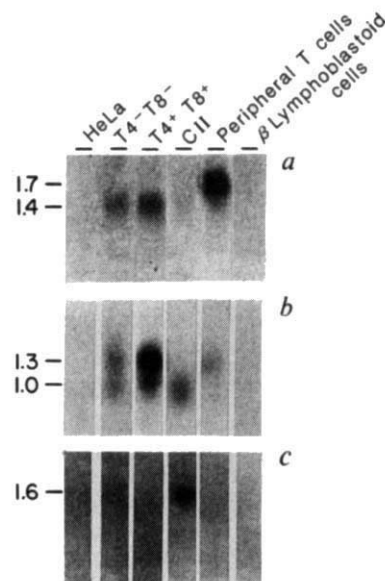


Fig. 4 Stage-specific expression of T-cell receptor (TCR) genes: *a*, α ; *b*, β ; *c*, γ . Approximately 6 μg of total RNA from each of the cell populations indicated was fractionated by formaldehyde/agarose gel electrophoresis, transferred to nitrocellulose or Nytran and assayed for hybridization to ^{32}P -labelled human α , β and γ cDNA probes (kindly provided by T.W. Mak and described elsewhere^{6,38,39}). Hybridization conditions were performed as described previously⁴⁰.

alone proliferated. Since anti-T3 is also known to induce cytotoxicity in normal T cells, we studied its effect on the induction of killing by CII cells²⁹. We found that the addition of OKT3 to CII induced killing of ^{51}Cr -labelled U937 macrophage targets (Fig. 3a). Untreated CII cells or CII cells treated with anti-T11 were not cytotoxic. In other experiments we found that anti-T3 induced CII cells to kill the natural killer cell target K562 (Fig. 3b). Taken together, these experiments demonstrate that antibody perturbation of the T3 molecule on CII results in the induction of immunological functions analogous to those observed in mature T lymphocytes.

Because the functional T3 molecule is physically associated with the T-cell receptor on mature T cells, it was of interest to determine whether the CII cells expressed mature α and β mRNA, and to determine whether CII expressed γ mRNA, known to be present in $\text{T4}^-\text{T8}^-$ murine thymocytes. Total RNA from CII cells as well as from thymocyte subpopulations ($\text{T4}^-\text{T8}^-$ and $\text{T4}^+\text{T8}^+$), mature T cells, B-lymphoblastoid cells and HeLa cells was assayed by Northern blotting procedures for hybridization to ^{32}P -labelled α -, β - and γ -specific complementary DNA probes (Fig. 4). HeLa cells and the lymphoblastoid cells did not express mature α , β or γ mRNA. In contrast, peripheral T cells, expressed the mature 1.7-kb α mRNA and a mature 1.3-kb β mRNA, but did not express γ mRNA¹³. Freshly isolated $\text{T4}^-\text{T8}^-$ cells expressed γ mRNA as well as both the 1.3-kb mature β mRNA and the 1.0-kb $D-J$ (diversity-joining regions) β transcript. In addition, these cells expressed an immature 1.4-kb α transcript but not the mature 1.7-kb α mRNA¹³. The $\text{T4}^+\text{T8}^+$ thymocyte population, thought to contain cells at a more advanced stage of development, did not express γ mRNA, but did express both the mature 1.3-kb β mRNA and to a lesser degree the 1.0-kb β transcript. We did not detect the 1.7-kb α mRNA in the $\text{T4}^+\text{T8}^+$ population, but observed only the immature 1.4-kb α transcript.

The CII cell line expressed high levels of γ mRNA but did not express the mature 1.3-kb β mRNA or the mature 1.7-kb α transcript. However, CII cells did express the 1.0-kb β transcript and a faint band between 1.0 and 1.4 kb

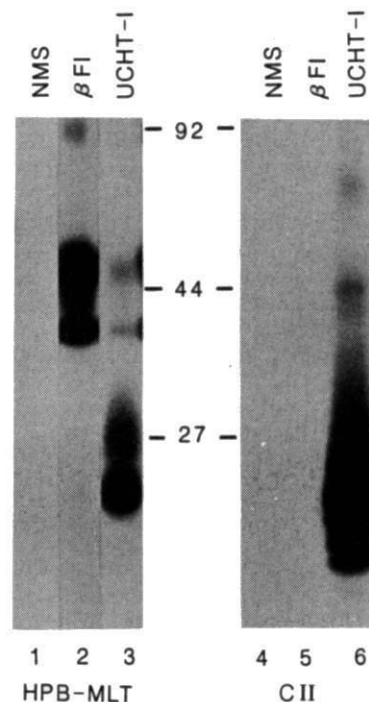


Fig. 5 SDS-PAGE analysis of T3 and T3-associated molecules from CII and HPB-MLT. ^{125}I -labelled detergent lysates from T-leukaemia cell line HPB-MLT and thymic clone CII were examined by SDS-PAGE. Immunoprecipitations were performed with nonspecific control antibody (normal mouse serum (NMS) in lanes 1 and 4, framework monoclonal antibody to the β -chain of the T-cell receptor (β F1) in lanes 2 and 5, and anti-T3 antibody (UCHT-1) in lanes 3 and 6 (ref. 29)).

Methods. ^{125}I -labelling was performed using the lactoperoxidase technique. Following radiolabelling, 10^7 lymphocytes were solubilized in Tris-buffered saline (pH 8) containing 1% Triton X-100. Lysates were immunoprecipitated using 1 μl NMS, 1 μg β F1 monoclonal antibody or 1 μg UCHT-1 monoclonal antibody. Immunoprecipitated proteins were resolved by SDS-PAGE (10.5% acrylamide) and radiolabelled species were visualized by autoradiography as described previously¹⁴.

which may correspond in part to the immature 1.4-kb T α transcript. Taken together, these data suggest a developmental progression of human T α , T β and T γ gene expression similar to that observed in murine thymocytes, with γ genes expressed earliest, followed by β and then α gene expression^{13,15-17}. In this light, CII cells represent an early stage of thymocyte development in which γ genes are expressed in the absence of mature α and β gene expression.

The above data demonstrate that T3 surface expression in CII occurs in the absence of the expression of mature T α or T β genes. Therefore, it was important to determine whether the T3 molecule on CII cells was physically associated with other surface proteins. For this, we immunoprecipitated ¹²⁵I-labelled surface proteins with anti-T3 as well as with antibodies to framework determinants of the β -chain (β F1)³⁰. Immunoprecipitated proteins were analysed by SDS-polyacrylamide gel electrophoresis (PAGE) under reducing conditions (Fig. 5). Immunoprecipitation of CII by anti-T3 revealed a T3 molecular complex (18–28K) that co-precipitated with two additional bands which migrated at 44K and 62K (Fig. 5, lane 6). In contrast, no bands were seen when CII was immunoprecipitated with β F1 (Fig. 5, lane 5). Immunoprecipitations of the control mature HPB-MLT cell line with anti-T3 revealed, as described previously, a T3 molecular complex co-precipitating with bands migrating at 40K and 49K (Fig. 5, lane 3)¹⁴. In addition, immunoprecipitation of HPB-MLT with β F1 revealed the 49K and 40K bands, representing the T α and T β chains¹⁴.

Our data demonstrate that the functional T3 molecule on the CII cells, although not associated with the $\alpha\beta$ heterodimer, is associated with a novel heterodimer of 44K and 62K. This structure is not seen on HPB-MLT cells (Fig. 5, lanes 2, 3) and has not been identified previously on human thymocytes or mature T cells. The finding of high levels of T γ mRNA expression in CII raises the possibility that one of the subunits of the 44/62K structure on CII could be encoded by the T γ gene. However, definitive designation of one of the subunits as a γ peptide must await amino-acid sequence analysis and comparison with deduced T γ protein sequence from CII T γ cDNA.

Surface expression of T3 is thought to mark a mature stage of T-cell development, and recent studies have suggested that surface T3 expression may depend on T α and T β expression^{26,31}. Our studies show definitively, however, that T3 can be expressed on immature thymocytes lacking T α and T β . Nevertheless, the concept that expression of surface receptor molecules may be essential for T3 surface expression may be correct, as CII cells do express a T3-linked 44/62K structure. Our studies, showing that antibodies to the T3 molecule associated with this heterodimer induce proliferation, IL-2 release and cytotoxicity, strengthen this idea by suggesting that the T3-44/62K complex on CII is functional. It has been speculated that precursor thymocytes may bear self-MHC receptors involved in the selection of the mature T-cell receptor repertoire intrathymically^{13,15,32,33}. If the CII cells described here do express self-MHC receptors, a candidate structure for this receptor is the T3-44/62K complex.

It is noteworthy that the 44/62K structure is not co-immunoprecipitated by anti-T3 antibodies from mature T cells¹⁴. This finding does not preclude its presence in a non-T3-linked form. Conceivably, mature T cells express a non-T3-linked 44/62K receptor, which might bind self-MHC molecules and participate in recognition of the antigen-MHC complex along with the T3-linked T-cell receptor. This hypothesis would permit independent interactions of the non-T3-linked 44/62K receptor with self-MHC. Such interaction would not necessarily induce autoreactivity because the T3 molecule may be essential for signal transduction²⁶.

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Note added in proof: We have now immunoprecipitated solubil-

ized and ¹²⁵I-labelled proteins from C2 using anti-T3 and the anti- γ -chain sera described by Brenner *et al.*³⁰. Immunoprecipitated proteins were analysed by SDS-PAGE under reducing conditions. The anti- γ sera identifies the T3-associated 44K protein in CII.

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Thy-1 functions as a signal transduction molecule in T lymphocytes and transfected B lymphocytes

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Thy-1, a glycoprotein of relative molecular mass 25,000 (25K), is a major constituent of the cell surface of mouse thymocytes, peripheral T cells and neurones¹. In man, Thy-1 is present on neurones and on a small percentage of thymocytes, but is absent from peripheral T cells². The amino-acid^{3,4} and complementary DNA^{5,7} sequences of Thy-1 indicate that it has a structure similar to an isolated V (variable region) domain of immunoglobulin⁴. Although the function of Thy-1 is unknown, the ability of different anti-Thy-1 monoclonal antibodies to activate murine T cells^{8,9} or induce functional changes in neuronal cells *in vitro*¹⁰ suggests that Thy-1 is involved in transmembrane signalling. We now show that crosslinking of murine Thy-1 triggers a rapid rise in the cytoplasmic free calcium concentration ($[Ca^{2+}]_i$), not only in murine T cells and Thy-1.2-transfected human T cells, but also in murine B-lymphoma cells transfected with the murine *thy-1.2* gene. These results indicate that the generation and transduction of the signal leading to the rise in $[Ca^{2+}]_i$ is independent of the T-cell receptor and other T-cell-specific molecules. The preservation of the $[Ca^{2+}]_i$ -

modulating function of Thy-1 in various lymphoid cells of two species further suggests that the necessary signal either originates in the Thy-1 molecule itself or is generated in concert with a highly conserved molecule(s) associated with Thy-1.

To define the cell surface and the intracellular requirements for signal transduction via Thy-1, several previously characterized cell lines which do not naturally express Thy-1, including the human T-cell tumour Jurkat E6.1 (ref. 11) and the murine B-lymphoma lines NBL.DUB¹², M12.4.1 (ref. 13) and WEHI 231 (ref. 14), were transfected with a murine genomic *thy-1.2* clone. In Fig. 1, the fluorescence observed after staining of the murine T-cell hybridoma P.4.4 (a) with the anti-Thy-1 monoclonal antibody 3H11 is compared with that seen after staining of the *thy-1.2*-transfected clones Jurkat J14C9 (b), NBL.23C5 (c), M12.46E5 (d) and WEHI.40C2 (e). Because volume measurements demonstrated a very similar cell size in the populations examined, the amount of Thy-1.2 expressed by the cells could be estimated from the degree of fluorescence observed. Compared with P.4.4 (100%), the highest levels of Thy-1 were found on NBL.23C5 (88.3%); lower amounts were seen on M12.46E5 (40.9%), J14C9 (31%) and WEHI.40C2 (8.9%). Although the *thy-1.2*-transfected cell clones were reactive with a large panel of anti-Thy-1 monoclonal antibodies, they failed to bind antibody G7, which is mitogenic for resting murine T cells⁸. The parental lines Jurkat E6.1, NBL.DUB, M12.4.1 and WEHI 231 showed no reactivity with any of the anti-Thy-1 antibodies tested (results not shown).

As a rapid rise in $[Ca^{2+}]_i$ is an early event in a large number of cell activation models¹⁵, including the activation of T cells¹⁶⁻²⁰, we measured $[Ca^{2+}]_i$ after crosslinking of Thy-1 on the surface of the transfected cell lines as well as on murine thymocytes, peripheral T lymphocytes and the T-cell hybridoma P.4.4. For these measurements we used the fluorescent indicator Quin-2, which is trapped intracellularly, binds Ca^{2+} with a 1:1 stoichiometry, and shows increased fluorescence on binding Ca^{2+} (ref. 21). The cells to be tested were first loaded with Quin-2, incubated with rat antibodies 3H11 and 3A7, which recognize independent epitopes on Thy-1 (ref. 22), and washed. The preincubation with the two monoclonal antibodies did not change the resting $[Ca^{2+}]_i$ (results not shown). The cells were then reacted with MAR 18.5, a mouse antibody to rat κ -chains which does not bind to human or murine T or B cells²³. Preincubation with two anti-Thy-1 monoclonal antibodies gave better quantitative results with thymocytes, normal T cells and the T-cell hybridoma P.4.4, whereas identical tracings were obtained when only one anti-Thy-1 monoclonal antibody was used for preincubation of Jurkat J14C9 and the B-cell lines NBL.23C5 and M12.46E5 (results not shown). The tracings of Quin-2 fluorescence observed after crosslinking of Thy-1 on the various cell populations are shown in Fig. 2. After the addition of MAR 18.5, a rapid rise in $[Ca^{2+}]_i$ was seen in murine thymocytes (Fig. 2a), normal T cells (b) and the T-cell hybridoma P.4.4 (c). A similar result was observed in the transfected human cell line Jurkat J14C9 (Fig. 2d). As J14C9 expresses the T3 complex, which is known to trigger a rise in $[Ca^{2+}]_i$ (ref. 18), we compared the effect of crosslinking Thy-1 with the effect of antibody OKT3 (ref. 24) in the Quin-2 assay; the response to OKT3 (Fig. 2e) was more rapid and resulted in a greater rise in $[Ca^{2+}]_i$, which then reversed more quickly.

When the Thy-1 molecule on the transfected B-cell lines was subjected to the same crosslinking procedure using monoclonal antibodies 3H11/3A7 and MAR 18.5, both NBL.23C5 (Fig. 2f) and M12.46E5 (Fig. 2g) demonstrated a rapid rise in $[Ca^{2+}]_i$. In contrast, WEHI.40C2 (Fig. 2h) failed to respond in analogous experiments. As a positive control, the addition of a purified anti-IgM antibody preparation, which is known to increase $[Ca^{2+}]_i$ by crosslinking surface immunoglobulin²⁵, gave a positive signal in all three transfected B-cell lines (Fig. 2k-m). In general, anti-IgM antibodies produced a higher initial rise in $[Ca^{2+}]_i$, but crosslinking of both surface immunoglobulin and

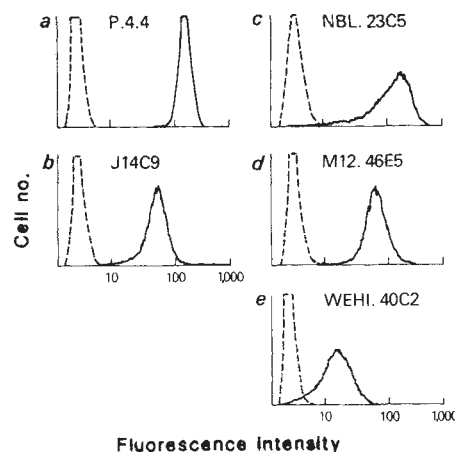


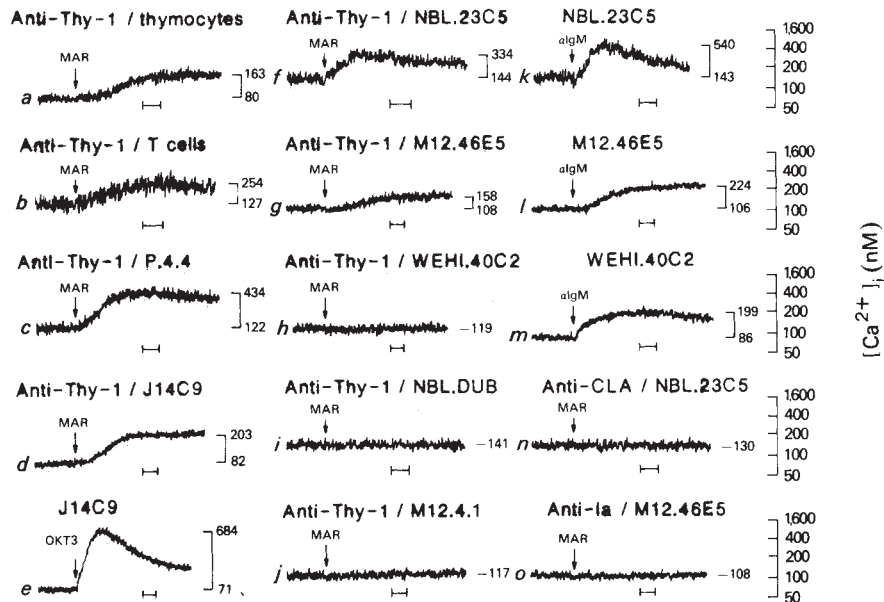
Fig. 1 Flow microfluorimetric analysis of Thy-1 expression on the T-cell hybridoma P.4.4 (a) and on the *thy-1.2*-transfected cell lines J14C9 (b), NBL.23C5 (c), M12.46E5 (d) and WEHI.40C2 (e). Cells (1×10^6) were preincubated with excess anti-Thy-1 antibody 3H11 (ref. 33) (—) for 30 min at 4°C, then washed three times with Hanks' balanced salt solution containing 0.1% NaN_3 and 3% fetal calf serum. The cells were then stained with excess fluorescein isothiocyanate (FITC)-conjugated antibody MAR 18.5, which is specific for rat κ light chains²³, and 3×10^4 viable cells (as determined by propidium iodide exclusion) were analysed on a fluorescence-activated cell sorter (FACS II: Becton Dickinson). The broken line indicates background fluorescence with FITC-MAR 18.5 alone. Control staining of the parental (Thy-1-negative) cell lines Jurkat E6.1, NBL.DUB, M12.4.1 and WEHI 231 yielded results identical to the FITC-MAR 18.5 background of the respective transfectants (results not shown). Measurements of cell volume were performed on a cell analyser (Becton Dickinson). Calculations indicated a cell-surface ratio for P.4.4/J14C9/NBL.23C5/M12.46E5/WEHI.40C2 of 1:1.41:0.93:0.96:0.85. The T-cell hybridoma P.4.4 was produced as described previously³⁴; *thy-1.2*-transfected cell lines were produced as described in detail elsewhere²⁷. Briefly, an 8.2-kilobase (kb) *EcoRI* fragment containing the complete *thy-1.2* gene was cloned into the *EcoRI* site of the pSV2gpt vector³⁵. Spheroplasts were produced from *Escherichia coli* HB101 and fused to Jurkat E6.1 and to the murine B-cell lines. Expression of the *Ecogpt* gene in transfected cells enabled selection and long-term maintenance of cells in the presence of mycophenolic acid, hypoxanthine and xanthine. Colonies which grew out of selection were cloned at limiting dilution and analysed for Thy-1.2 expression by flow microfluorimetry.

Thy-1 produced a similar sustained elevation of $[Ca^{2+}]_i$. The reason for the differential behaviour of WEHI.40C2 remains to be determined, but it is possible that the relatively low expression of Thy-1 on the surface of this cell line (see Fig. 1) prevented an effective crosslinking of the bound anti-Thy-1 monoclonal antibodies by MAR 18.5.

Several control experiments were performed in order to rule out nonspecific effects of the crosslinking procedure on $[Ca^{2+}]_i$. First, when the parental (Thy-1-negative) lines of the responsive transfected T- and B-cell lines were preincubated with antibody 3H11 or 3A7, washed, and then reacted with MAR 18.5, no increase in $[Ca^{2+}]_i$ was seen in NBL.DUB (Fig. 2i), M12.4.1 (Fig. 2j) or Jurkat E6.1 (result not shown). Second, a number of monoclonal antibodies to other cell-surface antigens present on the cell lines were subjected to crosslinking with MAR 18.5 in the Quin-2 assay; no elevation of $[Ca^{2+}]_i$ was seen in either NBL.23C5 preincubated with a monoclonal antibody to the common leukocyte antigen (CLA; Fig. 2n) or M12.46E5 preincubated with an anti-Ia monoclonal antibody (Fig. 2o). Negative results were also observed when NBL.23C5 was pretreated with a monoclonal antibody recognizing H-2, and when M12.46E5 was pretreated with antibodies to H-2, CLA or B220. Furthermore, negative results were obtained with P.4.4 after preincuba-

Fig. 2 Tracings of Quin-2 fluorescence representing $[Ca^{2+}]_i$ in Thy-1-bearing and *thy-1.2*-transfected cells. The scale for the ordinate has been standardized by appropriate optical reduction of the tracings; the scale bar in each record represents 1 min. α IgM, anti-IgM antibody.

Methods. In a modification of the method described by Tsien *et al.*²¹, the cells to be assayed ($5 \times 10^6 \text{ ml}^{-1}$) were loaded in culture medium with a final concentration of $15 \mu\text{M}$ Quin 2-AM for 20 min. After loading, the cells were washed twice with Dulbecco's phosphate-buffered saline, resuspended in the same buffer to a concentration of $5 \times 10^6 \text{ ml}^{-1}$ and used immediately after equilibration at 37°C for 5 min. Where indicated, Quin-2-loaded cells (1×10^7) were preincubated with monoclonal antibody ($250 \mu\text{l}$ of 1:10 ascites of $250 \mu\text{l}$ of purified antibody; 1 mg ml^{-1}) for 15 min at 37°C and washed twice more. Fluorescence intensity was measured with a Perkin Elmer fluorescence spectrophotometer LS-5 (excitation 339 nm, emission 492 nm). The cuvette chamber was kept at 37°C , and the cell suspension continuously stirred. After a baseline had been established, antibody MAR 18.5 (1:100 ascites) or OKT3 (1:100 ascites) or anti-IgM antibodies ($10 \mu\text{g ml}^{-1}$) were added. Maximum fluorescence (F_{max}) was determined by lysing the Quin-2-loaded cells with 0.1% Triton X-100. Minimum fluorescence (F_{min}) was obtained after addition of EGTA (4 mM) and sufficient Tris buffer to raise the pH to 8.3. Approximate values for cytoplasmic free Ca^{2+} were calculated using the formula: $[Ca^{2+}]_i = 115 \text{ nM} (F_x - F_{\text{min}})/(F_{\text{max}} - F_x)$ (ref. 21). The following antibodies were used for preincubation: 3H11 (Thy-1)³³, 3A7 (Thy-1)³³, M1.4/42.3.9.8 (H-2 common determinant)³⁶, M1/9.3.4.HL.2 (CLA)³⁶, FD441.8 (LFA-1)³⁷, M5/114 (Ia common determinant)³⁸ and RA3-3A1 (B220)³⁹. The affinity-purified anti-IgM antibodies (given by Dr Junichiro Mizuguchi) were prepared as described previously⁴⁰.



tion with antibodies to H-2, CLA and lymphocyte function antigen (LFA-1) (results not shown). Thus, numerous lymphocyte surface antigens failed to act like Thy-1 in inducing a rise in $[Ca^{2+}]_i$, demonstrating the specificity of the latter effect.

The ability of Thy-1 to act as a signal transducing molecule was first noted when it was recognized that anti-Thy-1 antibodies present in rabbit antisera to mouse brain were responsible for the T-cell mitogenic effects of such antisera²⁶. More recently, we and several other groups have confirmed and extended these studies by demonstrating that certain monoclonal anti-Thy-1 antibodies can activate resting murine T cells, as well as T-cell hybridomas and cytotoxic T-cell clones^{8,9}. Furthermore, we have shown recently that after crosslinking, a large number of unselected, non-mitogenic anti-Thy-1 antibodies (including 3H11 and 3A7) are capable of inducing a vigorous proliferative response in murine T cells and interleukin-2 (IL-2) production in *thy-1.2*-transfected human Jurkat clones in the presence of phorbol esters; IL-2 production could be observed in murine T-cell hybridomas even in the absence of a co-stimulator^{22,27}. Our previous results, together with the present data, demonstrate that in every case in which crosslinking of Thy-1 elicited a rise in $[Ca^{2+}]_i$, this was followed by functional responses in the T-cell populations studied.

The ability of crosslinked Thy-1 to trigger a rise in $[Ca^{2+}]_i$ in *thy-1*-transfected B cells clearly indicates that the signal delivered is independent of the T-cell antigen receptor and other T-cell-specific molecules. The preservation of the signalling function of Thy-1 in several lymphoid cell types of two species could be explained by a model in which Thy-1 associates, not only in murine B and T cells but also in human T cells and presumably in rodent and human neuronal cells, with a highly conserved molecule responsible for the rise in $[Ca^{2+}]_i$. Alternatively, the triggering signal leading to a rise in $[Ca^{2+}]_i$ may be generated by the Thy-1 molecule itself. Recent results indicate that Thy-1 is anchored directly to a cell membrane lipid, probably phosphatidylinositol^{28,29}. Whether this peculiar mode of membrane integration is related to the function of Thy-1 is at

present unknown, but it is interesting to note that breakdown products of membrane phosphoinositides are involved in receptor-mediated signalling events, including changes in $[Ca^{2+}]_i$ (reviewed in ref. 30).

The formulation of a hypothesis concerning the role of Thy-1 in the ontogeny and function of T cells is complicated by the differences in tissue distribution between species. The results of the present study are compatible with the view that Thy-1 has a critical role as an inducer of an alternative pathway of T-cell activation, in a manner similar to that proposed for the T11 antigen on human thymocytes and T cells^{31,32}. Thus, Thy-1 may represent a phylogenetically primitive pathway of cellular triggering which may have evolved prior to the antigen-specific receptor and which may function primarily in triggering thymocyte growth and differentiation. Whether this model is correct, and whether Thy-1 plays a similar part in transduction of intercellular differentiation signals in other Thy-1-bearing cell types, remains to be determined.

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Human T-cell γ genes contain *N* segments and have marked junctional variability

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The γ -chain genes are encoded by immunoglobulin-like gene segments in germline DNA which rearrange during the somatic development of T cells to form an active gene¹⁻⁷. The protein produced by these genes has not been identified and the diversity of the proteins that the genes can express has not been determined. We expect that the diversity of expressed γ -chains is produced by the same three mechanisms that produce diversity of other immunoglobulin-like genes: (1) germline variable (*V*) and joining (*J*) region repertoires; (2) somatic mutation; and (3) junctional diversity^{8,9}. To define the contribution of each of these mechanisms to the generation of γ -chain diversity, several γ -chain complementary clones and rearranged γ -chain genes have been characterized. Most of these clones seem to encode a defective γ -chain, the variable- and constant-region portions being joined such that they would not be translated in the same reading frame. Here we report that the germline *J*-region diversity of the human T-cell γ -chain is very limited and that somatic mutation does not contribute to the diversity of the γ -chains encoded by the cloned segments. However, the junctional diversity of these γ -chain genes is extensive. We suggest that *N* sequences (template-independent sequences) have been inserted enzymatically into all of the γ -chain genes characterized.

The repertoire of germline *V* and *J_γ* segments encoded in the human genome is small. Recent studies have demonstrated that the human genome contains 12 variable-region segments,

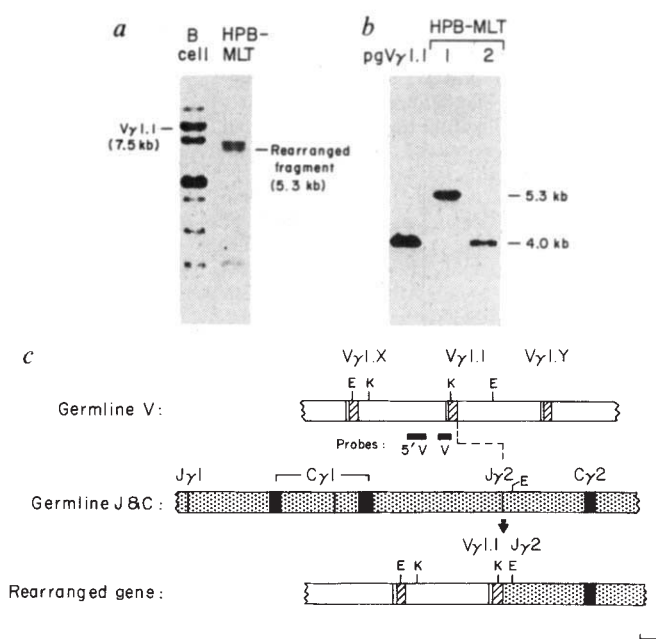


Fig. 1 Southern blots of germline and HPB-MLT $V_{\gamma 1}$ variable regions (*a*) and cloned $V_{\gamma 1.1}$ (pgV $\gamma 1.1$) and size-selected HPB-MLT genomic DNA (*b*). *a*, B-cell (Epstein-Barr virus-transformed B-cell line, LAZ 509) and HPB-MLT T-cell line DNA was digested with *EcoRI*, size fractionated on a 0.9% agarose gel, transferred to nitrocellulose and hybridized to a ³²P nick-translated variable-region probe from pT γ -1 (ref. 12). The rearranged fragment was identified by its hybridization to both *V* and *J* probes. Samples were run on the same gel, fragment sizes were determined by co-migration with γ DNA digested with *HindIII*. *b*, Plasmid pgV $\gamma 1.1$ contains a 7.5-kb *EcoRI* fragment which hybridizes to the *V* probe, and was cloned into SP6 from an EMBL 3A human placental library using standard techniques¹². HPB-MLT DNA was digested with *EcoRI* and size fractionated on a preparative 0.8% agarose gel¹²; the fraction which contained the rearranged fragment was identified by hybridization to a genomic *J* probe. pgV $\gamma 1.1$ DNA was digested with *KpnI* and electrophoresed on the same 0.9% agarose gel with the *EcoRI*-digested size-selected HPB-MLT DNA (lane 1) and the size-selected DNA digested with *KpnI* (lane 2). DNA was transferred to a nitrocellulose filter and hybridized to ³²P-labelled 5' *V* probe (*c*). *c*, Variable- and constant-region exon arrangement was determined by nucleotide sequence analysis and hybridization to the cDNA pT γ -1. Variable-region exons are shown by hatching, constant exons are solid, the constant locus of the chromosome is stippled. Probes 5' *V* and *V* were isolated by cleavage of pgV $\gamma 1.1$ (ref. 12). Abbreviations: E, *EcoRI*, K, *KpnI*. Scale bar, 1 kb.

making up four separate families (refs 6, 7 and W.S., T.Q. and J.G.S., unpublished results). At least six of the *V* segments can recombine with *J* segments encoded in the γ -chain constant-region locus (Fig. 1c and ref. 6). Two *J* segments were identified by restriction fragment analysis in the γ -chain locus. To determine the difference between the amino-acid sequences encoded by the two *J* segments and to define their contributions to the rearranged genes, we have determined their nucleotide sequences¹⁰. The *J* regions and 1,000 base pairs (bp) of flanking sequence were determined. The 49-bp *J* segments encode identical peptides (they differ by only 1 bp; Fig. 2b; ref. 7). Homology between the two loci extends outside the coding region with >95% homology over the 500 bp from the *J* segment in either direction. The finding of extensive homology between the human *J_{γ1}* and *J_{γ2}* indicates that the human constant-region locus has duplicated recently, since the divergence of mouse and human, and that the *J_γ* segment does not contribute to γ -chain diversity.

Fig. 2 Restriction map and DNA sequence of germline $V_{\gamma 1.1}$, $J_{\gamma 2}$ (also $J_{\gamma 1}$), and the rearranged V - J junction and N segment. **a**, Variable (▨) and joining segments (▩) are indicated in germline and rearranged configuration. The germline variable region and constant region (▩) are joined by somatic recombination. Open arrowheads represent recombination signals; the restriction fragments used for nucleotide sequence analysis are indicated by horizontal arrows. Abbreviations: S, *Sac*I; R, *Rsa*I; K, *Kpn*I; A, *Sau*3A; H, *Hind*III; O, *Dra*I; N, N segment. **b**, Complete sequence of $V_{\gamma 1.1}$, $J_{\gamma 1}$, $J_{\gamma 2}$ and V - J junction as determined from sequence analysis of pgV $\gamma 1.1$, pJ $\gamma 1$, pJ $\gamma 2$ and cDNA pT γ -1, respectively. Splice signals are indicated by dotted lines over appropriate nucleotides; recognition sequences for recombination are overlined. Nucleotides not encoded in germline V or J segments are boxed and labelled N. Scale bar in **a**, 100 bp.

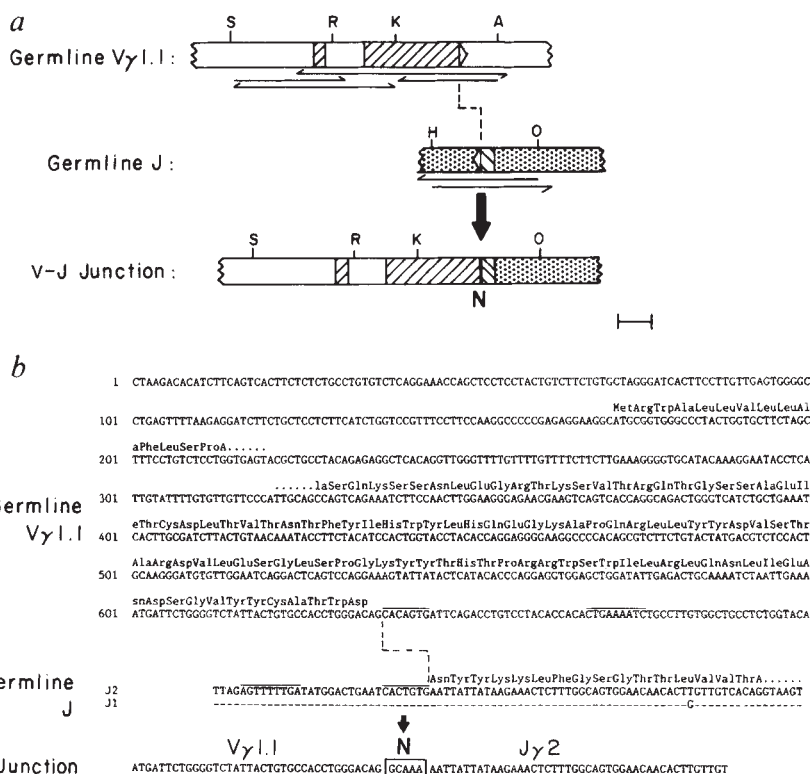


Fig. 3 Comparison of germline $V_{\gamma 3}$ and $J_{\gamma 2}$ sequence with the $V-J$ junction of cDNA pT γ -15. Heptamer and nonamer recombination sequences are overlined. Nucleotides not derived from germline sequence are boxed and labelled N. Vertical lines indicate identity of compared sequences.

GERMLINE V3: GluLysGluAspMetAlaValTyrTyrCysAlaAlaTyrTrpValAlaI
GAGAAAGAACATGGCCCTTTACTACTGTGTCGCGTGGTGGGCGACATATAGAAGCTGTTGAAACAACATGCACAAAATCCCTC

VJ JUNCTION: GAGAAAGAACATGGCCCTTTACTACTGTGTCGCGCGG **N** **GATTATCAG** TTATTATAAGAAACTCTTTGGCACTGGAAACAACACTGTTGTGCACA

GERMLINE J2: TTAGAGTTTTTGATATGGACTGAATCACTGCGAATTATTATAAGAAACTCTTTGGCACTGGAAACAACACTGTTGTGTGCACA
AsnTyrTyrLysLysLeuPheGlySerGlyThrThrLeuValValThr

The contribution of somatic mutation and junctional variation to the diversity of expressed γ -chains can be determined by comparing the nucleotide sequences of the germline *V* and *J* segments with the nucleotide sequence of the γ -chain messenger RNA in particular T cells. We have previously obtained a full-length cDNA clone, pT γ -1, which corresponds to γ -chain mRNA from the T cell HPB-MLT (ref. 11). Southern blot analysis of germline (B-cell) DNA using the variable-region probe obtained from this cDNA clone indicated that the human genome contains a family of eight hybridizing variable-region gene segments (Fig. 1a), all except two of which have been deleted from the HPB-MLT genome (Fig. 1a, HPB-MLT). Most of the germline variable-region segments were cloned by screening an EMBL3A human placental library using the pT γ -1 *V*-region probe (data not shown and ref. 12). To determine which of these variable-region fragments was joined to the *J* segment to form the rearranged gene expressed in HPB-MLT, we compared the restriction enzyme map 5' of the rearranged γ -chain gene with the restriction enzyme map 5' of the germline variable-region genes (Fig. 1a and our unpublished results). Only one germline variable-region gene segment (designated $V_{\gamma}1.1$) yielded a 4.0-kilobase (kb) *Kpn*I digestion fragment that hybridized to a 5' *V* probe and co-migrated with a fragment produced by *Kpn*I digestion of purified rearranged fragment (Fig. 1b). Thus, $V_{\gamma}1.1$ is the germline variable-region fragment that underwent recombination with *J* γ 2 to produce the rearranged gene expressed in HPB-MLT (Fig. 1c).

Any differences between the nucleotide sequence of V_γ1.1 and the variable region utilized in HPB-MLT either occurred

by somatic mutation or are caused by polymorphic differences between the two individuals from whom the genes were obtained. To determine the extent of the differences between the two genes, we have determined the nucleotide sequence of $V_{\gamma}1.1$ (Fig. 2a). We found that this germline variable-region gene is like other immunoglobulin and T-cell β -chain V segments and is encoded in two exons, a hydrophobic leader segment and a variable region. The conserved heptamer and nonamer sequences, thought to play an important part in somatic recombination and found 3' of all immunoglobulin-like V -region segments, are observed 3' of the $V_{\gamma}1.1$ variable region. The $V_{\gamma}1.1$ sequence was compared with the nucleotide sequence of the variable region expressed in HPB-MLT (Fig. 2b). Because there are no differences between the two variable-region genes, we conclude that somatic mutation has not occurred within this variable region since it underwent recombination to form the rearranged gene found in HPB-MLT. The sequence of a genomic rearranged γ gene utilizing this V region has been published recently and differs at a single nucleotide⁷. This difference could be a result of sequencing error, polymorphism or somatic mutation. If the difference is a product of somatic mutation, it is not important in generating γ -chain diversity, since it is a silent mutation and does not affect the γ -chain amino-acid sequence.

The availability of germline *V* and *J* sequences as well as the nucleotide sequence of the cDNA expressed from the rearranged gene allows us to examine the mechanisms that produce diversity at the *V-J* junction. Comparison of the germline *V_γ1.1* and *J_γ2* sequences with the HPB-MLT cDNA sequence (Fig. 2*b*) indicates that 5 bp have been inserted between *V* and *J* during

Fig. 4 Junctional variability of rearranged γ genes. pT γ -1, pT γ -10, pT γ -15 are cDNAs, pT γ R4 is a rearranged genomic clone. λ S γ 12 and λ K γ 20 are rearranged genomic clones described in ref. 7. The non-germline nucleotides (labelled N segment) of pT γ -1, λ S γ 12 and pT γ -15 were determined by direct comparison with germline sequence (Figs 2, 3 and ref. 11). The variable regions have been aligned at the second conserved cysteine codon and V γ 1.1 used as a guide to predict the 3' end of the variable regions in pT γ R4 and pT γ -10. A predicted N segment was thus derived for pT γ R4 where germline sequence was not available. pT γ -10 and λ K γ 20 use the same V and J region but the sequences differ at the V-J junction, indicating that at least one of them has an N segment. Since the germline sequence of this variable region is not known, pT γ -10 has been arbitrarily chosen to represent a complete V region and the 3' end predicted by comparison with V γ 1.1. This conservative analysis results in a 5-bp N segment in λ K γ 20. It is possible that pT γ -10 contains up to 9 bp of N sequence. The entire germline J sequence is encoded in pT γ -1; J-segment bases missing in the other clones are indicated by blank spaces.

REARRANGED GENE	VARIABLE REGION	N SEGMENT	JOINING REGION
pT γ -1 (HPB-MLT)	...GGG GTC TAT TAC TGT GCC ACC TGG GAC AG	GCAAA	AATTATTATAAGAACTCTTT...
λ S γ 12	...GGG GTC TAT TAC TGT GCC ACC TGG	CGGACG	AATTATTATAAGAACTCTTT...
pT γ -15 (HSB-2)	...GCC GTT TAC TAC TGT GCT GCG TGG	GATTATCAG	TTATTATAAGAACTCTTT...
PREDICTED N SEGMENT			
pT γ R4 (JURKAT)	...GTG GTG TAC CCA TGT GCC TGT CAG ATC CT	CACAGGGCGGGTT	TAAGAACTCTTT...
pT γ -10 (HSB-2)	...GCT ACC TAC TAC TGT GCC TTG TGG GAG GT	G	AATTATTATAAGAACTCTTT...
λ K γ 20	...GCT ACC TAC TAC TGT GCC TTG	CGAGG	TTATTATAAGAACTCTTT...

somatic recombination. New sequences of this type have been identified in rearranged immunoglobulin and T-cell receptor β -chain genes and result either from additional germline diversity (D) segments or sequences inserted enzymatically in a template-independent fashion (N segments)^{8,9}.

We have investigated whether there are additional base pairs between the V and J segments of other rearranged γ -chain genes by cloning a partial γ -chain cDNA (pT γ -15) from the T-cell line HSB-2, which would encode another defective γ -chain (Fig. 3). This cDNA contains a γ -chain V region which we have designated V γ 3 and which is a member of a different variable-region gene family (data not shown). The 3' end of the V γ 3 gene was cloned on a 9.5-kb EcoRI fragment isolated from a subgenomic library produced from size-selected Jurkat DNA. Nucleotide sequence analysis of the germline V γ 3 variable region and the HSB-2 cDNA (pT γ -15) identified no differences between the germline gene and the expressed cDNA in the coding portion. This sequence identity suggests that the rearranged γ -chain genes found in mature T cells have not been subjected to extensive somatic mutation. However, during the recombination that joined this V to J, 9 bp have been inserted between the V and J segments.

To determine whether additional sequences are inserted between the V and J sequences of other rearranged γ -chain genes, we have isolated a rearranged genomic γ -chain gene (pT γ R4) and another cDNA (pT γ -10). The germline V-region segments that were involved in the formation of these rearranged γ -chain genes have not been sequenced. However, we can predict the location of the 3' end of the germline variable region by aligning the codons encoding the conserved cysteine residues (Fig. 4). Thus, using V γ 1.1 as a guide, this analysis predicts the presence of a 13-bp additional sequence in one rearranged gene and a 1-bp addition in the other (Fig. 4).

The additional bases inserted between the V and J segments are probably added enzymatically during site-specific recombination and represent N segments. Evidence for a D segment between V and J segments in the immunoglobulin heavy-chain genes and the T-cell antigen receptor β -chain genes has been obtained by the identification of recombined D-J segments in plasmacytomas or T cells¹³⁻¹⁵. The human γ -chain J segments rearrange to produce six different variable regions⁶. In more than 50 T-cell lines and tumours (corresponding to more than 100 rearranged chromosomes) no other rearrangements that might represent D-J recombination have been detected (ref. 6 and J. Greenberg, T.Q., J.G.S. and J. Kersey, unpublished results). Although the exact origin of N segments is not known, a model for their generation has been proposed which involves terminal transferase^{16,17}. In other immunoglobulin-like gene rearrangements, N segments have been described only in association with D segments^{8,9}. Rearranged immunoglobulin light-chain genes seem to use neither D nor N segments. The human γ -chain gene provides the best evidence for an immunoglobulin-

like gene that does not have a D segment but contains N segments.

Rearranged murine γ -chain genes have also been identified which contain two or three additional base pairs between the V and J segments^{2,3,18}. Although it has been proposed that these additional base pairs may represent a D or N segment, there is no evidence for a separate D segment. Although both the murine and human rearranged γ -chains have additional nucleotides separating the V and J segments, the rearranged human genes have considerably longer N segments than the murine genes (Fig. 4). The 9- and 13-bp N segments found in rearranged human γ -chain genes are as long as the total sequence found separating many T-cell receptor β -chain and immunoglobulin V and J regions^{8,9}.

The human γ gene can further produce junctional diversity by altering the site of recombination in V- and J-region segments. Eight bases at the 3' end of the germline V γ 3 segment are absent in the rearranged gene (Fig. 3), and up to eight bases are lost from the 5' germline J sequence during recombination (Fig. 4). Flexibility in the site of recombination is thought to be an important source of variable-region diversity among other immunoglobulin-like genes and apparently occurs in the formation of rearranged γ -chain genes.

An important feature of all four rearranged γ -chain genes described here is that they are rearranged in a way that will not produce an active gene. However, Lefranc *et al.* have recently described two γ -chain genes that have rearranged so that they encode productive γ -chain transcripts⁷. Comparison with the germline variable-region sequences presented here suggests that both of these genes have N segments inserted between V and J segments (Fig. 4). The apparently high rate (four of six) of ineffective γ -chain gene rearrangement may represent the high price paid to achieve such flexibility of V-J joining. Presumably, the defective γ -chain genes rearranged in many mature T cells are not functional and these T cells do not have a requirement for functional γ -chain expression. One possibility is that junctional variability represents a by-product of a mechanism of recombinational inactivation of the γ -chain gene; that is, the γ -chain genes might rearrange during development of the T cell to produce a functional γ -chain and subsequently undergo a second rearrangement as a feature of terminal T-cell differentiation. We have noted previously the infrequent use of C γ 1 in T cells, and we suggest that functional rearrangements of J γ 1 occur in early T cells and may be lost during somatic development.

The human γ -chain derives little combinatorial diversity from two virtually identical J segments, although the possibility of further diversity resulting from rearrangement to other J-C loci has not been excluded. There seems to be little somatic mutation in the genes that have been sequenced. However, significant variability is introduced at the V-J junction by N segments and variation in the V and J recombination site. These mechan-

isms appear to be more active in the human γ -chain locus than in the murine locus. Although the mechanisms by which *N* segments are inserted into the *V-J* junction are unclear, this region in immunoglobulin proteins plays a significant part in determining antigen-binding specificity¹⁹. Thus, we believe that the junctional variability of the γ -chains will have an important role in determining their function in the immune response.

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Newly replicated DNA is associated with DNA topoisomerase II in cultured rat prostatic adenocarcinoma cells

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DNA topoisomerases have been proposed to function in a variety of genetic processes in both prokaryotes and eukaryotes¹⁻³. Here, we have assessed the role of DNA topoisomerase II in mammalian DNA replication by determining the proximity of newly synthesized DNA to covalent enzyme-DNA complexes generated by treating cultured rat prostatic adenocarcinoma cells with teniposide. Teniposide (VM-26), an epipodophyllotoxin, is known to interact with mammalian DNA topoisomerase II so as to trap the enzyme in a covalent complex with DNA⁴⁻⁹. We have found that the teniposide-induced trapping of such complexes requires $MgCl_2$, is stimulated by ATP and is inhibited by novobiocin. The formation of covalent complexes seems to be reversible on removal of teniposide. Furthermore, analysis of the covalent complexes formed between ³H-thymidine pulse-labelled DNA and topoisomerase II following teniposide treatment reveals a direct association of the enzyme with nascent DNA fragments. Our results suggest that DNA topoisomerase II may interact with newly replicated daughter DNA molecules near DNA replication forks in mammalian cells.

We assayed for the appearance of teniposide-induced covalent complexes between topoisomerase II and newly replicated DNA in cultured Dunning R3327-G rat prostatic adenocarcinoma cells. For this we used a potassium-sodium dodecyl sulphate (K-SDS) precipitation procedure specific for protein-linked DNA^{9,10}. Monolayer cultures of growing cells were pulse-

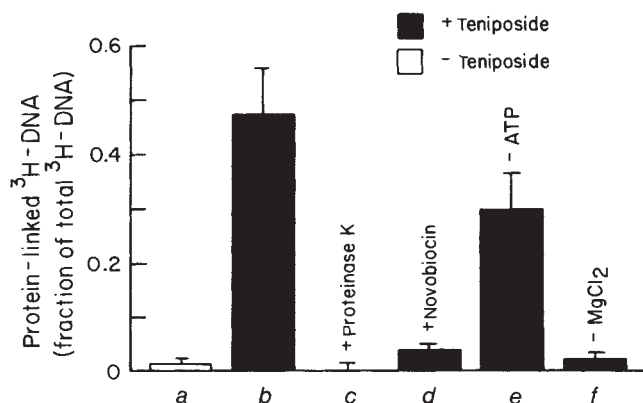


Fig. 1 Covalent linkage of pulse-labelled DNA to protein following teniposide treatment requires an activity characteristic of mammalian topoisomerase II. The fraction of pulse-labelled ³H-DNA linked to protein (protein-linked ³H-DNA/total ³H-DNA) was determined for: a, control, lysates from cells incubated in buffer I without teniposide; b, teniposide, lysates from cells incubated with 20 μ M teniposide; c, lysates from teniposide-treated cells that had been digested with 200 μ g ml⁻¹ proteinase K for 30 min at 50 °C; d, lysates from cells incubated with teniposide in buffer I supplemented with 2 mM novobiocin; e, lysates from cells incubated with teniposide in buffer I without ATP; f, lysates from cells incubated with teniposide in buffer I without $MgCl_2$. Protein-linked ³H-DNA fractions are expressed as means $\pm$ s.e.m.

Methods. Monolayer cultures of growing Dunning R3327-G rat prostatic adenocarcinoma cells were pulse-labelled for 90 s at 37 °C by exposure to 20 μ Ci ml⁻¹ ³H-thymidine (54 Ci mmol⁻¹) in complete medium (RPMI 1640 supplemented with 10% fetal calf serum, 25 nM dexamethasone, 100 U ml⁻¹ penicillin, 100 U ml⁻¹ streptomycin). Immediately after pulse labelling, the cells were permeabilized *in situ* by gently washing first for 5 min, and then again for 10 min at 4 °C with buffer E (0.5% Nonidet P-40, 10% glycerol, 10 mM NaCl, 5 mM $MgCl_2$, 1 mM EGTA, 1 mM phenylmethylsulphonyl fluoride in 10 mM sodium phosphate, pH 6.5). In preparation for teniposide treatment, the cells were rinsed twice for 5 min at 4 °C with buffer I (100 mM NaCl, 5 mM $MgCl_2$, 1 mM EGTA, 0.1 mM dithiothreitol, 0.5 mM ATP in 10 mM sodium phosphate, pH 6.5), buffer I without $MgCl_2$, buffer I without ATP, or buffer I supplemented with 2 mM novobiocin. The rinsed, permeabilized cells were then incubated for 30 min at 37 °C in the same buffer with 0.2% DMSO (a) or with 20 μ M teniposide and 0.2% DMSO (b-f) before being lysed in lysis buffer (2% SDS and 10 mM EDTA in 10 mM Tris, pH 8.0). The lysates were subjected to the K-SDS assay^{9,10}. Briefly, for the non-denaturing K-SDS assay, 500 μ l of 120 mM KCl were added to an equal volume of SDS lysate. The mixture was heated to 65 °C for 10 min, cooled at 4 °C for 5 min and then centrifuged at 1,500g for 5 min at 4 °C. The K-SDS-protein precipitate was recovered and washed by resuspending the precipitate in 1 ml of wash buffer (100 μ g ml⁻¹ calf thymus DNA, 20 μ g ml⁻¹ bovine serum albumin, 100 mM KCl, 1 mM EDTA in 10 mM Tris pH 8.0) and heating at 65 °C for 10 min. The wash mixture was then cooled at 4 °C for 5 min and centrifuged at 1,500g for 5 min at 4 °C. After five washes, the amount of protein-linked ³H-DNA was determined by dissolving the final precipitate in 1 ml of water before counting in 10 ml of Aquasol-2 (NEN). The total amount of ³H-DNA was determined by assessing the amount of trichloroacetic acid-insoluble ³H-DNA in the lysates.

labelled for 90 s with ³H-thymidine; in order to limit the ³H-thymidine pulse duration to 90 s, the cells were placed on ice and permeabilized immediately after labelling. The permeabilized cell monolayers were then treated *in situ* with teniposide in the absence of exogenous deoxynucleoside triphosphates before being lysed in SDS. When SDS lysates from these cell monolayers were subjected to the K-SDS assay, ³H-DNA was recovered along with the K-SDS precipitate (Fig. 1b). Digestion of the lysates with proteinase K reduced the amount of K-SDS-precipitable ³H-DNA by >99% (Fig. 1c), confirming the specificity of the K-SDS assay for protein-linked DNA. Markedly less topoisomerase II-linked ³H-DNA was detected when the topoisomerase II inhibitor novobiocin was included during teniposide treatment (Fig. 1d), while omission of teniposide (Fig. 1a), ATP (Fig. 1e) or $MgCl_2$ (Fig. 1f) from the incubation mixture resulted in a reduced amount of enzyme-linked ³H-DNA, as expected for a topoisomerase II reaction^{4-6,8,10}.

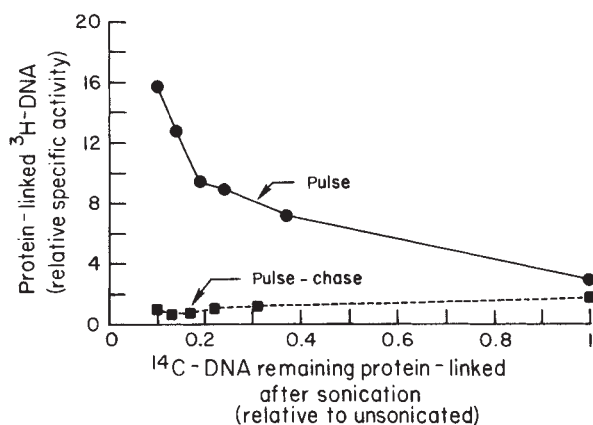


Fig. 2 Enrichment of newly replicated DNA with teniposide-induced covalent DNA-protein complexes. Cultured Dunning R3327-G rat prostatic adenocarcinoma cells that had been labelled for 72 h with $0.05 \mu\text{Ci ml}^{-1}$ ^{14}C -thymidine (60 mCi mmol^{-1}) were incubated with $20 \mu\text{Ci ml}^{-1}$ ^3H -thymidine ($54 \mu\text{Ci mmol}^{-1}$) in complete medium for 90 s at 37°C (pulse, ●) or exposed to $20 \mu\text{Ci ml}^{-1}$ ^3H -thymidine for 90 s and then incubated in $40 \mu\text{M}$ unlabelled thymidine in complete medium for 45 min at 37°C (pulse-chase). Both pulse and pulse-chase labelled cells were permeabilized, treated with teniposide and lysed in lysis buffer as described for Fig. 1. Then, in order to progressively reduce the size of DNA remaining protein-linked, the lysates were sonicated for 0, 5, 10, 15, 20 and 30 s with a Branson Model W140 Sonifier at setting 2 before being subjected to the K-SDS assay as for Fig. 1. The specific activity of ^3H -DNA in protein-linked DNA relative to the specific activity of ^3H -DNA in total DNA [(protein-linked ^3H -DNA c.p.m./protein-linked ^{14}C -DNA c.p.m.)/(total ^3H -DNA c.p.m./total ^{14}C -DNA c.p.m.)] was determined. This relative specific activity was plotted as a function of ^{14}C -DNA remaining protein-linked after sonication (normalized to the unsonicated protein-linked ^{14}C -DNA value).

Furthermore, protein-linked ^3H -DNA complexes could be reversed by $>60\%$ after incubating the teniposide-treated monolayers in the absence of teniposide for 4 h at 37°C . The time dependence of reversal (data not shown) was similar to that observed using purified topoisomerase II⁵.

^3H -thymidine pulse and pulse-chase labelling experiments revealed that newly replicated DNA was specifically enriched within the teniposide-induced topoisomerase II-DNA complexes. Briefly, monolayer cultures that had been labelled previously for 72 h with ^{14}C -thymidine were incubated with ^3H -thymidine for 90 s (pulse) or exposed to ^3H -thymidine for 90 s and then incubated with a 100-fold excess of unlabelled thymidine for 45 min (pulse-chase). Immediately following pulse or pulse-chase labelling, the cell monolayers were permeabilized, treated with teniposide, and then lysed in SDS. At this point, the SDS lysates were sonicated for various lengths of time in order to progressively reduce the average fragment size of DNA remaining covalently bound to topoisomerase II. The topoisomerase II-linked DNA fragments in the sheared lysates were then recovered using the K-SDS precipitation procedure. The results of these experiments (see Fig. 2) indicated that as the amount of K-SDS-precipitable ^{14}C -DNA was reduced by sonication, protein-linked DNA from pulse-labelled cells became progressively enriched in ^3H -DNA (that is, the specific activity of protein-linked ^3H -DNA relative to the specific activity of total ^3H -DNA became >1). In contrast, protein-linked DNA from pulse-chase labelled cells was not enriched in ^3H -DNA following sonication. In the absence of teniposide treatment, neither pulse-labelled nor pulse-chase labelled cells displayed any enrichment of ^3H -DNA among protein-linked DNA, whether sonicated or not.

We believe that the association of pulse-labelled DNA with topoisomerase II-DNA covalent complexes may reflect a functional interaction of topoisomerase II with newly replicated DNA. However, another possible explanation for the observed

enrichment of newly replicated DNA with teniposide-induced topoisomerase II-DNA covalent complexes is that DNA at the replication fork might be more readily accessible for spurious binding to abundant nuclear proteins like topoisomerase II than is bulk nuclear DNA. We therefore treated pulse-labelled permeabilized cells with camptothecin, a plant alkaloid reported to trap covalent mammalian DNA topoisomerase I-DNA complexes¹¹, and found that camptothecin generated covalent protein-DNA complexes but failed to reveal any enrichment of ^3H -DNA among protein-linked DNA after sonication.

Furthermore, the enrichment for pulse-labelled ^3H -DNA with topoisomerase II-linked DNA was not limited to our permeabilized cell system. We found that for short ^3H -thymidine labelling times, intact cells exposed to teniposide also contained protein-linked DNA enriched in ^3H -DNA following sonication (Table 1). Cell monolayers that had been labelled previously with ^{14}C -thymidine were incubated with ^3H -thymidine for 5, 10, 20 and 30 min. In order to trap topoisomerase II-DNA complexes in the intact cells, teniposide was added to the ^3H -thymidine incubation medium for the final 5 min of ^3H -thymidine labelling. When the cells were lysed in SDS and sonicated, topoisomerase II-linked DNA enriched in ^3H -DNA was recovered along with the K-SDS precipitate (see Table 1). The enrichment for ^3H -DNA was greater for shorter ^3H -thymidine labelling times.

Table 1 Teniposide treatment of intact cells reveals an enrichment of newly replicated DNA with covalent protein-DNA complexes

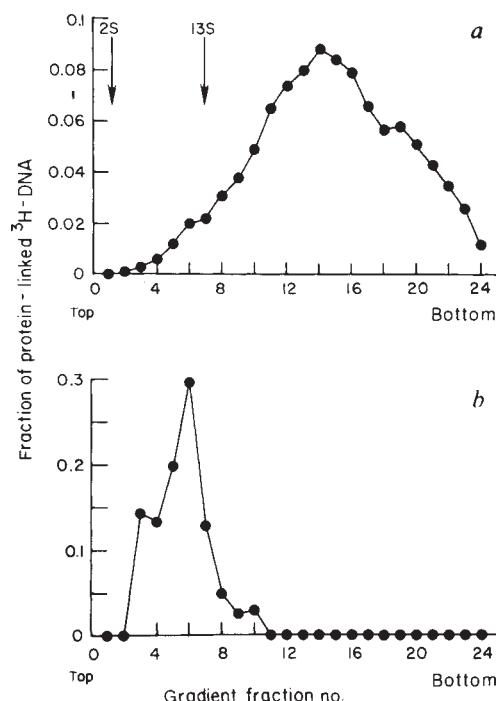
^3H -thymidine labelling time (min)	Protein-linked ^3H -DNA (relative specific activity)
5	19.1 ± 0.3
10	11.3 ± 0.3
20	7.7 ± 0.7
30	6.4 ± 0.1

Cultured Dunning R3326-G rat prostatic adenocarcinoma cells that had been labelled for 72 h with 0.05 Ci ml^{-1} ^{14}C -thymidine (60 mCi mmol^{-1}) were incubated with 20 Ci ml^{-1} ^3H -thymidine (54 Ci mmol^{-1}) in complete medium for 5, 10, 20 or 30 min at 37°C . To trap topoisomerase II-DNA covalent complexes, teniposide was added to the ^3H -thymidine-containing medium to a final concentration of $20 \mu\text{M}$ (0.2% dimethyl sulphoxide, DMSO) for the final 5 min of ^3H -thymidine labelling. Following ^3H -thymidine labelling and teniposide exposure, the cell monolayers were lysed directly in SDS lysis buffer and the SDS lysates were sonicated for 30 s with a Branson Model W160 Sonifier before being subjected to the K-SDS assay described in Fig. 1 legend. The specific activity of ^3H -DNA in protein-linked DNA relative to the specific activity of ^3H -DNA in total DNA was determined as for Fig. 2 and is expressed as the mean $\pm$ s.d. The ^3H -thymidine labelling time dependence of this relative specific activity is given.

Topoisomerase II forms covalent complexes with DNA in the presence of teniposide such that each subunit (mammalian topoisomerase II is a homo-dimer) can be found in covalent linkage through a tyrosine to a 5'-phosphate on the cleaved substrate DNA^{5,10}. We sought to determine whether teniposide-induced covalent protein-DNA complexes were formed exclusively with unreplicated parental DNA near the replicating fork or whether nascent daughter DNA strands could be isolated with the complexes. In order to detect teniposide-induced complexes between topoisomerase II and nascent daughter DNA strands, ^3H -thymidine pulse and pulse-chase labelled cells were permeabilized, treated with teniposide and lysed in SDS (as in Fig. 2). The SDS lysates were sonicated and then analysed by using the K-SDS assay under both denaturing (single-stranded DNA; lysates heated to 100°C for 10 min) and non-denaturing (double-stranded DNA) conditions. We predicted that if covalent complexes were trapped near DNA replication forks, but only on unreplicated parental DNA, we should not detect denatured DNA enriched in ^3H -DNA in sonicated lysates from pulse-labelled cells. We found instead that when lysates from

Fig. 3 Nascent DNA strands can be isolated in covalent linkage with topoisomerase II following teniposide treatment. Following sucrose gradient centrifugation, the fractions of the total protein-linked ^3H -DNA (72-h label, *a*) and newly synthesized DNA (90-s label, *b*) recovered from each gradient were plotted as a function of fraction number (from the top). Arrows in *a* denote the position of the 2S (28 base pair, bp) and 13S (2,657 bp) markers determined by analysing aliquots from each fraction for ^{32}P c.p.m.

Methods. Dunning R3327-G rat prostatic adenocarcinoma cells growing in culture were labelled with $20\ \mu\text{Ci ml}^{-1}\ ^3\text{H}$ -thymidine ($54\ \mu\text{Ci mmol}^{-1}$) for 90 s or with $1\ \mu\text{Ci ml}^{-1}\ ^3\text{H}$ -thymidine for 72 h in complete medium at 37°C . Permeabilized monolayers treated with $20\ \mu\text{M}$ teniposide (as for Fig. 1) were lysed in 1 ml of alkaline lysis buffer (0.2 M NaOH, 0.2 M NaCl, 10 mM EDTA). The alkaline lysates were carefully applied to 30-ml 5–20% sucrose gradients on top of 5-ml 50% sucrose cushions in alkaline buffer (0.1 M NaOH, 0.9 M NaCl, 10 mM EDTA) and centrifuged in an SW27 (Beckman) rotor for 15 h at 4°C at 25,000 r.p.m. For DNA size markers plasmid pLY-1, a 2,679-bp pBR322 derivative, was digested with *EcoRI* and *Clai*, and then end-labelled by fill-in with $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ in the presence of unlabelled dGTP, dCTP and TTP using the Klenow fragment of DNA polymerase I (ref. 19), generating ^{32}P -labelled 28- and 2,657-bp DNA fragments. $s_{20,w}$ Values for the fragments under alkaline sedimentation conditions were estimated to be 2S (for the 28-bp fragment) and 13S (for the 2,679-bp fragment), respectively²⁰. The labelled fragments were applied as internal markers in the gradient containing the alkaline lysate from the 72-h-labelled cells. After centrifugation 1.5-ml fractions were collected from the top of each centrifuge tube by displacement with 70% sucrose. Fractions were neutralized by the addition of 0.1 ml 1.5 M HCl in 0.4 M Tris (pH 8.0). After the addition of 0.25 ml of 2×SDS lysis buffer and 0.25 ml of 240 mM KCl, 0.5-ml aliquots from each fraction were subjected to the K-SDS assay for protein-linked DNA as described for Fig. 1 (with two washes).



teniposide-treated pulse-labelled cells were sonicated for 30 s (as in Fig. 2), the denaturing K-SDS assay revealed an enrichment of ^3H -DNA among topoisomerase II-linked single-stranded DNA (13.7 ± 1.3 -fold) similar to that detected with the non-denaturing K-SDS assay for topoisomerase II-linked double-stranded DNA (13.4 ± 1.3 -fold). Sonicated lysates from pulse-chase labelled cells failed to display any enrichment for ^3H -DNA among topoisomerase II-linked DNA, regardless of whether the K-SDS assay was run under denaturing or non-denaturing conditions.

Further evidence that topoisomerase II might form covalent complexes directly with nascent DNA fragments in the presence of teniposide was provided by alkaline sucrose density gradient sedimentation studies. Pulse-labelled or uniformly labelled cells were permeabilized, treated with teniposide, dissolved in NaOH and then applied to alkaline sucrose gradients (see Fig. 3). After centrifugation, the gradient fractions were subjected to the K-SDS assay for protein-linked DNA. Similar to the findings of Chen *et al.*⁵, we found that most topoisomerase II-linked ^3H -DNA from uniformly labelled cells treated with teniposide sedimented to a size of 25–35S (Fig. 3*a*). Topoisomerase II-linked ^3H -DNA from pulse-labelled cells treated with teniposide sedimented to a size ($<12\text{S}$) characteristic of nascent DNA chains (Fig. 3*b*).

Our results indicate that mammalian DNA topoisomerase II can be isolated in covalent linkage with newly replicated DNA molecules near the site of DNA synthesis, following teniposide treatment of permeabilized cultured rat prostatic adenocarcinoma cells. The close proximity of the teniposide-induced covalent complexes to the replicating fork suggests that DNA topoisomerase II may be actively involved in the replication of mammalian cell DNA. These findings are consistent with the identification of topoisomerase II as a component of the mammalian replisome¹², a multi-enzyme complex responsible for DNA replication^{12,13}, and with the demonstration of topoisomerase II within the nuclear matrix¹⁴, the subcellular site for eukaryotic DNA synthesis¹⁵. An increase in topoisomerase II activity in rat liver following partial hepatectomy further supports a role for topoisomerase II in mammalian DNA replication¹⁶.

Previous studies on the role of DNA topoisomerase II in eukaryotic DNA replication have emphasized the importance of a topoisomerase II double-strand DNA passage activity in resolving the products of DNA synthesis prior to mitotic segregation¹⁷. However, our observation that mammalian DNA topoisomerase II covalent complexes may be located near the replicating fork suggests that the enzyme may also function during replication fork progression in mammalian cells. Cairns¹⁸ recognized the need for an effective swivel in his description of the replication of covalently closed DNA molecules. We speculate that a mammalian DNA topoisomerase II, located near the replication fork in association with daughter DNA molecules, might be able to remove parental helical turns that have been passed through the fork to the daughter strands.

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Nuclear magnetic resonance imaging of a single cell

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Nuclear magnetic resonance (NMR) imaging^{1,2} is now an established tool in clinical imaging and competes favourably with conventional X-ray computerized tomography (CT) scanning³. The drive behind NMR imaging has primarily been in the area of whole-body imaging, which has been limited clinically to fields of up to 1.5 T (60 MHz). It is recognized that there may be substantial advantages in obtaining images with sub-millimetre spatial resolution^{4,5}. Also, there may be benefits to imaging at higher fields, since the signal increases as the square of the magnetic field⁶. Using a modified 9.5 T 89-mm-bore high-resolution NMR spectrometer, we have now obtained the first NMR images of a single cell, demonstrating the advent of the NMR imaging microscope. The NMR microscope is expected to have considerable impact in the areas of biology, medicine and materials science, and may serve as a precursor to obtaining such resolutions on human subjects.

The spectrometer was equipped with a set of magnetic field gradient coils with corresponding control units and power supplies capable of producing gradients for spatial delineation in excess of 20 G cm⁻¹. In addition, a radiofrequency (r.f.) modulator, a linear amplifier, waveform memories for gradient and r.f. control, and image display facilities were interfaced with the spectrometer. Radiofrequency excitation and reception were performed using a 5-mm horizontal solenoid. The studies presented here used a spin-echo imaging technique^{7,8}. The delay between the excitation and the acquisition of the spin-echo (TE) and the time between excitation pulses (TR) are given for each image in the figure legends.

The cells used in these studies were ova obtained from *Xenopus laevis* (African clawed toad) by standard surgical extraction techniques⁹. The ova were mechanically stripped of ovarian tissue and maintained in Barth's solution, and ova in different stages of oogenesis were placed in a 1.1-mm (inside diameter) tube and imaged. Figure 1 shows that the nucleus is clearly resolved, with heterogeneity in the cell cytoplasm seen as two regions of differing intensity corresponding to the animal and vegetal poles of the ovum. The differences in image intensity result from proton density variations, differences in relaxation times, or a mixture of the two. The water in the cell cytoplasm is distinct from the free water surrounding the cell, as has been suggested by some investigators¹⁰. The water signal from the nucleus is distinct from that of the cytoplasm and is indistinguishable from that of the free water surrounding the cell, suggesting that nuclear water is less strongly bound than cytoplasmic water. The signal-to-noise ratio of these images indicates that a greater spatial resolution can be obtained in similar times. To date, a resolution of 10×13 μm with a 250-μm slice width has been achieved (Fig. 2), although greater resolutions are expected to be possible. The nucleus of the ovum in Fig. 2 is clearly resolved, and a greater signal is obtained from the animal pole of the ovum than from the vegetal pole. Such data are obtained in acceptable experimental times (see Figs 1–4) and can be directly related to morphological regions within the cell.

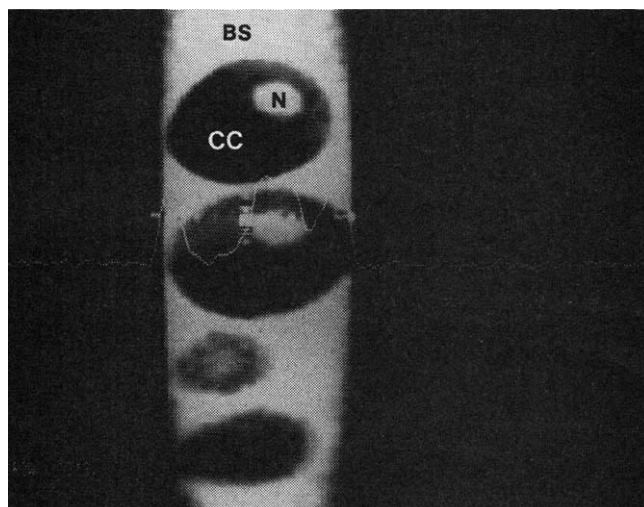


Fig. 1 NMR image of four ova from *Xenopus laevis* at different stages of oogenesis. The image represents a slice 500 μm thick lengthwise through the tube containing the intact ova. The image matrix is 256×256, which corresponds to a spatial resolution of 16×27 μm. Zero-filling was not used in any of the following images as a means of improving resolution because we noted no significant improvement in image quality. A spin-echo pulse sequence was used with a TE of 16 ms and a TR of 1 s, resulting in an imaging time of 4 min and 16 s. The image shows the clear distinction between the cell nucleus (N) and the cell cytoplasm (CC). The profile across one of the cells demonstrates a signal-to-noise ratio for the nucleus of ~15:1. The cell was surrounded by Barth's solution (BS).

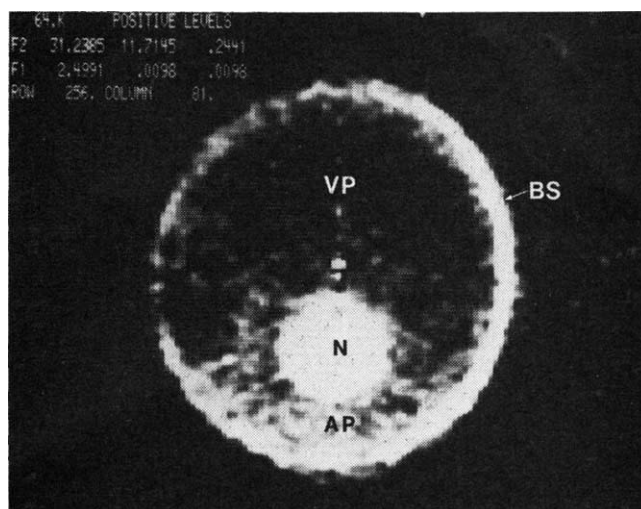


Fig. 2 NMR image representing a transverse slice across a glass tube containing a stage 4 *X. laevis* ovum; it has a spatial resolution of 10×13 μm and a slice width of 250 μm. The spin-echo pulse sequence used a TE of 16 ms, a TR of 4 s, and produced a data array of 128×128. Four experiments were averaged and thus the total imaging time was 32 min and 8 s. Although the image is relatively grainy, the differences between the cell nucleus and different zones of the cytoplasm are easily discerned. VP, vegetal pole; AP, animal pole.

We have also been able to infer information about the chemical composition of the cell. A chemical shift artefact in the form of an adjacent, slightly overlapping image was noted in early cell experiments (Fig. 3). This artefact slowly disappeared as the cells remained in the imaging system for longer periods of time. Earlier data¹¹ imply that this artefact is due to

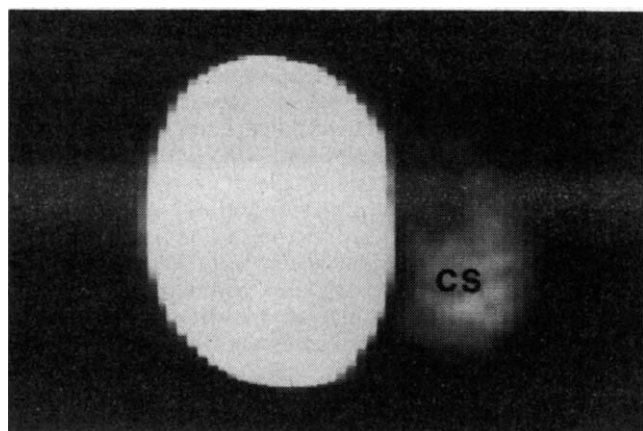
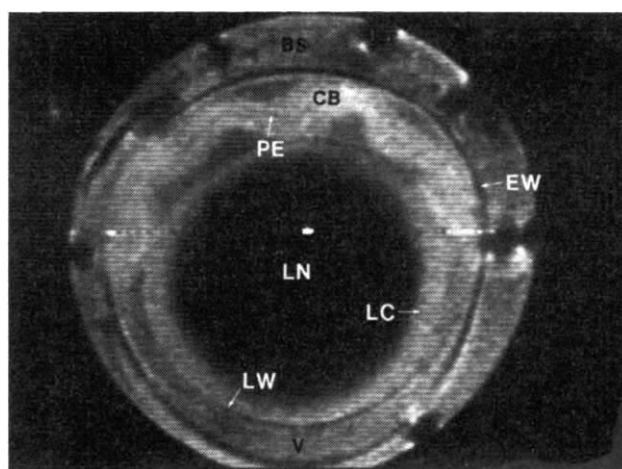


Fig. 3 (Left) NMR image showing a transverse section of a cell in a glass tube. The image acquisition parameters were the same as those of Fig. 1. The display constraints were adjusted so that low signal intensities can be easily observed. Under these display conditions the water signal from the cell appears as a solid white disk. To the right of this disk is a chemically shifted image (CS), which corresponds to the signal arising from the lipid content of the cell. The small dark area at the top of the chemical shift corresponds to the nucleus, indicating that there is little lipid within the nucleus.

Fig. 4 (Right) NMR micrograph of a mouse eye. The 256×256 data array has an in-plane resolution of $16 \times 27 \mu\text{m}$ and a slice width of $250 \mu\text{m}$. Only one data acquisition was used, with a TE of 16 ms and a TR of 2 s, resulting in an imaging time of 8 min and 32 s. The eye was placed in buffer solution within a 4.1-mm capillary tube. Considerable care was taken to ensure that there were no air bubbles around the eye, as these considerably distort the image. The image, from bottom to top, corresponds roughly to the eye, from side to side, although the presence of only one part of the ciliary body indicates that the imaging plane is tilted slightly from this equatorial plane. BS, buffer solution; LN, lens nucleus; LW, lens wall; LC, lens cortex; EW, eye wall; V, vitreous; CB, ciliary body. The dark patches in the surrounding buffer solution are due to extra-ocular material that remained after extraction.



the presence of lipid in the cytoplasm. Further studies should confirm the temporal dependence of this lipid. We speculate that the cell metabolizes these lipids during the experiment.

Figure 4 shows an image of a mouse eye to illustrate the clarity of images obtained at these high field strengths. The eye (~ 3.4 mm in diameter) was freshly extracted from a CD albino mouse. Structures within the eye are sharply resolved at a resolution of $16 \times 27 \mu\text{m}$, and the image definition is comparable to that of conventional NMR images. The resolution of this image is less than that of the ovum image in Fig. 2 because the object is larger; a larger matrix and hence a longer imaging time would have been required to obtain an image of the entire object. However, there is sufficient signal to obtain better resolutions on such samples, since both eye and cell images were obtained with an r.f. coil of the same diameter. The use of a zoom imaging technique should allow imaging of a small section of an object at high resolutions¹².

The slice widths within these images are considerably larger than the in-plane spatial resolution, primarily to reduce imaging times and improve the signal-to-noise ratio in the image. We

anticipate being able to use much smaller slice widths with these resolutions by efficient use and balance of imaging delays, r.f. coil design, imaging technique and higher fields. We note that in optical transmission microscopy the 'slice width' is determined by the physical width of the sample, which is often fixed, sectioned and stained. NMR imaging can be performed on intact living systems. No sectioning, fixing or staining was required to produce these NMR images and thus they are not subject to artefacts inherent in these procedures.

As expected, because the samples we studied were small, we observed no r.f. penetration problems. Also, no alterations in morphology were seen at this high field. Whether attenuation of r.f. energy at these frequencies will be a problem for larger samples and whether the high fields used are indeed harmful to living systems is unknown. Even if this should prove to be the case, we anticipate that a small-bore NMR microscope, as demonstrated here, will in itself prove to be a valuable tool in many spheres of science.

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Cephalosporin antibiotics can be modified to inhibit human leukocyte elastase

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Several laboratories, including our own, have reported the synthesis and activity of certain low relative molecular mass inhibitors of mammalian serine proteases¹⁻⁴, especially human leukocyte elastase (HLE, EC 3.4.21.37), an enzyme whose degradative activity on lung elastin has been implicated as a major causative factor in the induction of pulmonary emphysema⁵, and which is present in the azurophil granules of human polymorphonuclear leukocytes (PMN). Normally, these granules fuse with phagosomes containing engulfed foreign material (such as bacteria), and HLE, in combination with other lysosomal enzymes, catabolizes the particles^{6,7}. Under certain pathological conditions, however, PMN become attached to host protein (elastin fibres, basement membrane, connective tissue, immune complexes)^{8,9}, and in response to this adherence, the granules may fuse with the PMN outer membrane and release their contents, including HLE, directly onto the tissue¹⁰. Besides emphysema, HLE may also contribute to the pathogenesis of disease states such as adult respiratory distress syndrome¹¹, and its potential involvement in rheumatoid arthritis¹² makes HLE inhibitors of considerable interest. It is known that cephalosporin antibiotics (for example, cephalothin (compound I, Table 2)) are acylating inhibitors of bacterial serine proteases which help synthesize the cell wall by performing a transpeptidation reaction on a peptidyl substrate bearing a D-Ala-D-Ala terminus¹³. We now report that neutral cephalosporins (that is, compounds not bearing a free carboxyl at position C-4) can be modified to become potent time-dependent inhibitors of HLE.

Cephalosporins bearing substituents in the 7 α position are potent inhibitors of HLE (Table 2; details of the chemical synthesis of these compounds will be reported elsewhere). The corresponding 7 β isomers are either weaker inhibitors or inactive. The preference for α over β substituents is perhaps due to the fact that HLE cleaves L-L-amino acid peptide linkages whereas the target enzymes for β -lactam antibiotics cleave D-D-amino-acid peptide bonds, and thus 7 α -substituted cephe-

may be better mimics of mammalian substrates. Further, we have found that the enzyme prefers smaller substituents in the 7 α position, possibly because HLE cleaves proteins preferentially at sites where the P-1 (ref. 14) position is occupied by a relatively small alkyl substituent¹⁵. In the case of the alkyl ether series (compounds XIII $\rightarrow$ XIV $\rightarrow$ XV $\rightarrow$ XVI, Table 2), a rapid decrease in activity with increasing length of the alkyl group is observed. Branched groups (XVII) are also less active. The unsubstituted analogue (X) is inactive, but the 7 α -fluoro derivative (XXI) is highly active, indicating that the inductive nature of the substituent has a strong effect on the rate of acylation. That inductive effects are not of overwhelming importance, however, is amply demonstrated by the fact that the 7 α -ethyl analogue (XI) is a very potent inhibitor of the enzyme, almost as active as the isosteric 7 α -methoxy derivative (XIII). Finally, aromatic rings can be tolerated by the enzyme; the 7 α -phenyl analogue (XXIII) is roughly equipotent as an inhibitor to XIII, and in the ether series both XVIII and XX (but curiously not XIX) gave good inhibition of the enzyme.

The effects of modification of other parts of the cephalosporin nucleus have also been examined. With respect to the oxidation state of the sulphur, we have found that sulphones are generally the most active members of a series, with the (S)-sulphoxides and sulphides usually being an order of magnitude less active, and the (R)-sulphoxides being characteristically the least active of the series (compare compounds XIII $\rightarrow$ XXV $\rightarrow$ XXIV $\rightarrow$ XXVI and XIV $\rightarrow$ XXVIII $\rightarrow$ XXVII $\rightarrow$ XXIX; Table 2).

Modification of the functionality at position C-4 has been studied extensively (compounds XXXI-XXII; Table 2). As noted earlier, the free acids are generally poor inhibitors of HLE. In fact, carboxylates even more remote from the nucleus (for example, compound XXXIX) also result in a loss of inhibitory activity. Considering that HLE is an endopeptidase that cleaves peptide linkages between non-ionic amino acids, the enzyme preference for neutral molecules (at least those not bearing a charge in close proximity to the active site) is understandable. Decreasing the steric bulk of the ester (XIII $\rightarrow$ XXXIII $\rightarrow$ XXXII $\rightarrow$ XXXI) leads to some increase in activity. Incorporation of a benzene ring into the ester moiety (XXXIV) leads to a >10-fold enhancement in activity over XIII. Here again, placing a carboxyl at the C-4 position of the benzyl group yields a compound which is ~10-fold less active (XXXV), although still possessing substantial activity. The N-benzyl amide analogue (XXXVII) is ~100 times less active than the benzyl ester, but conversion of this secondary amide to a tertiary amide (XXXVIII) almost completely restores the inhibitory capacity of the molecule, leading to the interesting hypothesis that the *s-cis* amide conformation is required to fit the benzyl group appropriately into the active site. Note also that even a simple hydrogen at the C-4 position (XLI) still gives an entity with some inhibitory capability.

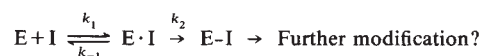
Most of the potent inhibitors in Table 2 cause time-dependent inhibition of HLE. (In Table 2 the compounds are evaluated according to their ability to cause 50% reduction in enzyme activity after an incubation period arbitrarily chosen to be 2 min. Greater inhibition is observed at longer times.) Preliminary kinetic data are consistent with a mechanism involving reversible complex formation followed by acylation of an active-site residue to produce the inactivated species (scheme 1). This

Table 1 Kinetic constants for inactivation of HLE by substituted cephalosporins

Compound	K_i (M)	k_2 (s ⁻¹)	k_2/K_i (M ⁻¹ s ⁻¹)
XIII	1.2×10^{-6}	0.023	19,000
XXII	1.8×10^{-7}	0.029	161,000

Rates of reaction versus time were measured at constant substrate and enzyme concentration, but varying inhibitor concentration. Data were computer fitted to the non-linear regression progress curve absorbance = $V_s t + (V_0 - V_s)(1 - e^{-kt})/k + C$ (where V_0 = initial velocity, V_s = final velocity, k = observed rate constant, t = time and C = constant of integration), which assumes first-order enzyme inactivation. K_i and k_2 were calculated from a reciprocal plot of k versus inhibitor concentration.

Scheme 1

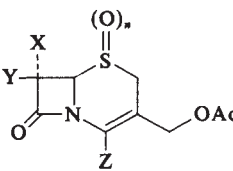


Acylated
enzyme

$$K_i = k_{-1}/k_1$$

scheme is similar to those describing the initial steps in the

Table 2 Activity of substituted cephalosporins against HLE

					
Compound No.	X	Y	Z	n	IC ₅₀ (μg ml ⁻¹)
I	H	ThCH ₂ CONH*	CO ₂ H	0	>20
II	H	ThCH ₂ CONH	CO ₂ C(CH ₃) ₃	0	>20
III	H	ThCH ₂ CONH	CO ₂ C(CH ₃) ₃	2	>20
IV	H	CH ₃ CONH	CO ₂ C(CH ₃) ₃	2	>20
V	CH ₃ CONH	H	CO ₂ C(CH ₃) ₃	2	>20
VI	H	CF ₃ CONH	CO ₂ C(CH ₃) ₃	2	>20
VII	CF ₃ CONH	H	CO ₂ C(CH ₃) ₃	2	2
VIII	H	HCONH	CO ₂ C(CH ₃) ₃	2	15
IX	HCONH	H	CO ₂ C(CH ₃) ₃	2	2
X	H	H	CO ₂ C(CH ₃) ₃	2	>20
XI	CH ₂ CH ₃	H	CO ₂ C(CH ₃) ₃	2	1
XII	H	OCH ₃	CO ₂ C(CH ₃) ₃	2	>20
XIII	OCH ₃	H	CO ₂ C(CH ₃) ₃	2	0.5
XIV	OCH ₂ CH ₃	H	CO ₂ C(CH ₃) ₃	2	1.5
XV	O(CH ₂) ₂ CH ₃	H	CO ₂ C(CH ₃) ₃	2	20
XVI	O(CH ₂) ₃ CH ₃	H	CO ₂ C(CH ₃) ₃	2	>20
XVII	OCH(CH ₃) ₂	H	CO ₂ C(CH ₃) ₃	2	>20
XVIII	OC ₆ H ₅	H	CO ₂ C(CH ₃) ₃	2	0.8
XIX	OCH ₂ C ₆ H ₅	H	CO ₂ C(CH ₃) ₃	2	>5†
XX	OCH ₂ CH ₂ C ₆ H ₅	H	CO ₂ C(CH ₃) ₃	2	2.5
XXI	F	H	CO ₂ C(CH ₃) ₃	2	0.03
XXII	Cl	H	CO ₂ C(CH ₃) ₃	2	0.02
XXIII	C ₆ H ₅	H	CO ₂ C(CH ₃) ₃	2	0.4
XXIV	OCH ₃	H	CO ₂ C(CH ₃) ₃	0	5
XXV	OCH ₃	H	CO ₂ C(CH ₃) ₃	1 (S)	2
XXVI	OCH ₃	H	CO ₂ C(CH ₃) ₃	1 (R)	>20
XXVII	OCH ₂ CH ₃	H	CO ₂ C(CH ₃) ₃	0	>20
XXVIII	OCH ₂ CH ₃	H	CO ₂ C(CH ₃) ₃	1 (S)	17
XXIX	OCH ₂ CH ₃	H	CO ₂ (CH ₃) ₃	1 (R)	>20
XXX	Cl	H	CO ₂ C(CH ₃) ₃	0	0.5
XXXI	OCH ₃	H	CO ₂ CH ₃	2	0.08
XXXII	OCH ₃	H	CO ₂ CH ₂ CH ₃	2	0.07
XXXIII	OCH ₃	H	CO ₂ CH(CH ₃) ₂	2	0.3
XXXIV	OCH ₃	H	CO ₂ CH ₂ Ph	2	0.03
XXXV	OCH ₃	H	CO ₂ CH ₂ PH(4-CO ₂ H)	2	0.2
XXXVI	OCH ₃	H	CON(CH ₃) ₂	2	0.6
XXXVII	OCH ₃	H	CONHCH ₂ Ph	2	2
XXXVIII	OCH ₃	H	CON(CH ₃)CH ₂ Ph	2	0.06
XXXIX	OCH ₃	H	CONHCH ₂ CO ₂ H	2	>20
XL	OCH ₃	H	CONHCH ₂ CO ₂ C(CH ₃) ₃	2	0.9
XLI	OCH ₃	H	H	2	2
XLII	OCH ₃	H	CO ₂ H	2	>20

HLE was isolated as described previously^{2,3}. Purified HLE was assayed spectrophotometrically at 25 °C by continuous monitoring of the release of *p*-nitroaniline from Boc-Ala-Ala-Pro-Ala-*p*-nitroaniline at 410 nm. Incubation mixtures contained inhibitor, 0.2 mM substrate (both added in dimethyl sulphoxide; DMSO), DMSO (10% final concentration) and enzyme (added last) in 0.05 M trimethylaminoethanesulphonic acid, pH 7.5. The reaction was followed for 10 min and the rate after 2 min (*r*) was determined from the slope of the progress curve at that time. Per cent inhibition was determined by $100 \times [1 - (r_{\text{inhibitor present}}/r_{\text{inhibitor absent}})]$. IC₅₀ (concentration of inhibitor giving 50% inhibition) was determined from a plot of % inhibition versus log inhibitor concentration. Except for compounds which gave less than 50% inhibition at 20 μg ml⁻¹, all inhibitors were studied over a concentration range that produced 15–90% inhibition.

* Th, 2-thienyl.

† The compound is insoluble at higher concentrations.

inactivation of β-lactamases by various β-lactams^{16,17}. Table 1 shows kinetic constants for inactivation by compounds XIII and XXII. Both compounds are potent inhibitors (*K_i* values micromolar or less which rapidly and irreversibly inactivate the enzyme). Preliminary data suggest that certain other β-lactams may function solely as competitive (reversible, non-time-dependent) inhibitors of HLE. Further mechanistic details are being examined. Experiments are also under way to elucidate the structural features of β-lactams which are responsible for rapid inactivation.

Table 3 shows the relative inhibition of several different serine proteases by a representative set of cephalosporins. Most of the compounds in this series are excellent inhibitors of porcine pancreatic elastase (PPE) as well as HLE, and to a lesser extent α-chymotrypsin. This series shows no significant activity against trypsin or human cathepsin G, although some activity is seen against human thrombin, and one compound (XVIII) shows trace activity against plasmin. Interestingly, compound XX shows unusual activity against chymotrypsin, implying that an appropriate chain length has been achieved to allow the phenyl

Table 3 Selectivity of modified cephalosporins against various serine proteases IC₅₀ (µg ml⁻¹)

Compound	HLE	PPE	ChT	Cat G	Trypsin	Plasmin	Thrombin
XIII	0.5	<0.1	7-8	>50	>50	>20	6
XXIV	5	15	>20	>50	>20	n.d.	n.d.
XXV	2	7	>20	>20	>20	>20	>20
XIV	1.5	0.5	10	>20	>20	>20	>20
XVIII	0.8	5	5	>20	>20	20	1
XX	2.5	0.5	<0.5	>20	>20	>20	>20

Both elastases were assayed with 0.2 mM Boc-Ala-Ala-Pro-Ala-*p*-nitroanilide as described in Table 1 legend. Other proteases were assayed similarly using Ac-Ala-Ala-Pro-Phe-pNA (chymotrypsin), Cbz-Gly-Pro-Arg-pNA (trypsin and thrombin) and tosyl-Gly-Pro-Lys-pNA (plasmin) (all at 0.2 mM). Cathepsin G was assayed with 0.2 mM Boc-Tyr-*p*-nitrophenyl ester in 0.05 M PIPES buffer, pH 6.5, containing 10% DMSO at 25 °C by measuring the production of *p*-nitrophenol at 348 nm. IC₅₀ values were obtained as described for Table 1. HLE and cathepsin G (Cat G) were prepared as described previously^{2,3}. Porcine pancreatic elastase (PPE) was obtained from Sigma Biochemicals, bovine α -chymotrypsin (ChT) and bovine trypsin were obtained from Worthington, and human thrombin and human plasmin were obtained from Boehringer Mannheim. n.d., not determined.

group to bind in the S₁ enzyme binding site.

One biological effect of elastase inhibitors is illustrated by the following observations. Microvascular haemorrhage can be part of an inflammatory response when PMN infiltrate inflamed tissue^{18,19}. Stetson and Good²⁰ postulated that this vascular damage may result from release of PMN proteases during phagocytosis. Argenbright *et al.*²¹ have shown that microvascular haemorrhage follows the intradermal injection of soluble human PMN granule contents into rabbits, and that this haemorrhage is dependent on HLE activity. Table 4 demonstrates that this

Table 4 Inhibition of microvascular haemorrhage by compound XIII

	Haemorrhage		<i>In vitro</i> activity	
	Blood vol./ skin site(µl)	% Inhibition	% Inhibition	
PMN extract +DMSO	86.5 ± 12	—	—	
PMN extract +100 µg XIII	0.8 ± 1.6	98	100	
PMN Extract +2.8 µg XIII	22.1 ± 10	75	84	
PMN extract +1.5 µg XIII	73.7 ± 18	14	25	

Intradermal microvascular haemorrhage was produced in rabbit skin by the injection of components derived from human PMN granules. Haemorrhage was quantitated by measuring the 30-min accumulation of ⁵⁹Fe-autologous red blood cells in the injected skin sites. The data are expressed as the change in blood content as a result of PMN extract injection (for example, the 5.0 ± 1.9 µl blood per site measured in vehicle injected sites has been subtracted from all other sites). Inhibition of haemorrhage was obtained by adding XIII directly to the PMN extract before injection. All sites received 0.4% DMSO. *In vitro* activity was determined by assaying a sample of PMN extract with and without addition of XIII against synthetic substrate according to previously published procedures¹⁵.

haemorrhage is inhibited by compound XIII, and further, that inhibition of haemorrhage correlates well with inhibition of HLE activity as measured *in vitro*.

Our study is the first reported demonstration that the appendages of a β -lactam nucleus, which has proved to be such an important source of bacterial enzyme inhibitors, can be modified to produce compounds that are potent inhibitors of human serine proteases. The implications of this work for further enzyme inhibitor design are clear, and we will shortly be reporting on the mechanism of this inhibition as well as our results with other β -lactam nuclei.

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